invivoscribe®

Personalized Molecular Medicine®

2018 PRODUCT CATALOG

Standardized solutions for the diagnosis, stratification, monitoring & study of oncology

Industry Pioneers

We pride ourselves on staying at the forefront of new technologies. We offer a full range of standardized CE-marked *in vitro* diagnostic products for detection of hematological malignancies, as well as RUO assays, analyte specific reagents, and nucleic acid controls.

Our LymphoTrack® Dx and LymphoTrack® Assays for use on both the Illumina® MiSeq® and Thermo Fisher Scientific® Ion PGM™ instruments can be used to identify clonal lymphocyte populations, track minimal residual disease, and evaluate the status of somatic hypermutation using our comprehensive, easy-to-use bioinformatics software.

Our CE-IVD gold-standard B- and T-cell PCR-based gel and capillary testing reagents, developed under ISO 13485 design control, are standardized, molecular hematopathology products that reduce discordance between laboratories and increase sensitivity and specificity.

Global Reach

The Invivoscribe portfolio of cGMP products are currently being used by more than 700 clinical and research laboratories across 105 countries worldwide.

Letter to Our Colleagues

Dear Colleagues:

I am pleased to introduce our 2018 Product Catalog and I would like to take this opportunity to share some 2017 developments and future plans at Invivoscribe.

In April of 2017 we received FDA approval of our PMA for the LeukoStrat® CDx *FLT3* Mutation Assay, a companion diagnostic that aids in the selection of patients with AML for whom midostaurin treatment is being considered. We were pleased to see successful culmination of the work we did for our partner, Novartis, and confirmation of the capabilities of our excellent team. In addition, we have now validated and released a CE-marked IVD version of this assay for use outside North America (page 62), and we are working towards FDA approval of a distributable version of this assay, so that we can offer it as a kitted product to our customers here in the US.

In 2017 we also released the LymphoTrack® *TRB* Assay. We now provide a comprehensive menu of LymphoTrack® Clonality Assays along with comprehensive interpretative LymphoTrack® software for the MiSeq® next-generation sequencing (NGS) platform (page 20). We will release our *TRB* Assay for Thermo Fisher® NGS platforms later this year. LymphoTrack® assays are used to identify and track clonal rearrangements within the antigen receptor loci (*IGH, IGK, TRG, TRB*). The 24 ID format allows customers to significantly reduce costs, as you can run any combination of the 7 assays (up to 154 samples and 14 controls) simultaneously, while the accompanying bioinformatics software automatically sorts and interprets the data for each targeted region and sequence ID.

Our team has enjoyed significant satisfaction in seeing our Research Use Only (RUO) assays being used in research and clinical trials in the US, and our CE-marked IVD versions of the assays being used by leading cancer centers abroad. These assays assist in the diagnosis and tracking of a wide variety of hematologic malignancies, and they are also used to evaluate somatic hypermutation (SHM) in chronic lymphocytic leukemia (CLL). In fact, a group at Oxford has already published a paper in Leukemia, describing use of our *IGHV* SHM Assay and our LymphoTrack® bioinformatics software in order to update prognostic stratification of patients with CLL.

These clonality assays will be submitted for review via the US FDA's 510(k) process. Additionally, our LymphoTrack® assays are also being used in concert with accompanying minimal residual disease (MRD) bioinformatics software, for the study of MRD in a wide range of hematologic malignancies, including multiple myeloma and acute lymphoblastic leukemia.

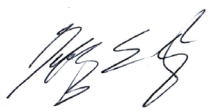
Our number of international clinical trials have expanded for one of our other pharmaceutical partners, Astellas, and we developed, validated and have started monitoring clinical trial subjects using our internationally standardized and CLIA/CAP-listed *FLT3*-ITD MRD assay that may be up to two logs more sensitive than other *FLT3* assays. These MRD assays and bioinformatics software are being used as a tool to support clinical validation endpoints in drug development studies for a number of pharmaceutical partners. We expect a paper describing the use of this assay to be published soon.

In our continuing efforts towards achieving international harmonization of molecular diagnostic testing, we are developing and will soon release a series of LymphoQuant™ MRD controls that can be used as internal spike-in controls with all of our MRD assays to help leading cancer research centers quantify residual disease. Further, our LymphoTrack® assays identify many CAR-T and engineered T cell constructs, so in a research or clinical trial setting they can be used throughout treatment to simultaneously identify and track both the subject's immune response and levels of immuno-therapy constructs.

To accommodate our rapid growth, Invivoscribe has moved into a brand-new, custom-designed facility in San Diego, more than doubling our previous company headquarter's space. We increased staffing in our San Diego (USA) and Martinsried (Germany) ISO 15189 accredited clinical laboratories, allowing us to provide clinical testing services 6 days a week. We set up a clinical laboratory in Japan, where we have started clinical testing and enrollment of subjects for clinical trials. In addition, we also opened an office in Shanghai (Invivoscribe Diagnostic Technology Co. Ltd.), to support clinical trials in China.

We have also expanded our NGS portfolio, developing and launching several NGS-based panels, including MRD testing for a number of hematologic biomarkers. Our MyAML®, MyMRD™, and MyHeme® gene panels, combined with our custom bioinformatics pipeline, MyInformatics™, are offered as RUO only and/or as CLIA/CAP-listed tests through our clinical laboratories in the USA, Europe and Asia.

Our continued growth and progress would not be possible without your constant feedback and support. We look forward to continuing our interaction with the research and clinical communities so we can offer you the best molecular products for decades to come. We wish you, your colleagues, and your families a joyful, productive, and successful 2018.



Jeffrey E. Miller, PhD
Founder, Chief Scientific & Chief Executive Officer, and Chairman

Table of Contents

Background

PCR & NGS-Based Assessment of Clonality in Hematologic Malignancies

For more than 20 years, Invivoscribe has developed, manufactured, and commercialized the gold-standard molecular hematopathology assays and reagents for gel and capillary electrophoresis detection, and most recently, next-generation sequencing instruments. These standardized, cGMP manufactured assays and reagents were developed and validated using standardized workflow and optimized primer sets, reagents and controls.

A number of our products were developed in collaboration with studies conducted by the EuroClonality BIOMED-2 concerted action and these capillary based products have provided reliable methods for clonality detection that have withstood the test of time.

Importantly, we have never accepted the status quo, so our comprehensive menu of clonality assays continues to evolve. All of our NGS-based clonality assays were developed in-house together with accompanying bioinformatics software by our Invivoscribe R&D team. Developed under full ISO 13485 design control, these assays and bioinformatics software were designed to run on several next-generation sequencing platforms. These NGS-based assays are several generations ahead of capillary-based products.

Our comprehensive bioinformatics software not only provides critical information on the presence of clonality, but also identifies the sequence information required to track clones in subsequent samples.

The unique process of gene rearrangement that occurs within the immunoglobulin (*Ig*) and T-cell receptor (*TCR*) gene loci during immune cell development and maturation generates a vast pool of genetically distinct cells. The resulting diverse population of lymphocytes displays an astonishing number of diverse antigen receptors, each coded in the DNA by a unique sequence, and each displayed on the cell surface, or as antibodies in the blood unique to a given cell [1,2]. This diversity allows the adaptive immune system to carry out its role in protecting the human body by recognizing the infinite number of pathogens it might encounter during a lifetime.

In sum, lymphoid malignancies are characterized by size- and sequence-specific rearrangements within these loci, which result from the transformation and subsequent proliferation from a single cell. The associated cellular population typically shares one or more cell-specific or "clonal" antigen-receptor gene rearrangements. The detection of these clonal cells forms the basis for clonality assessment in leukemia, lymphoma, and hematologic disease. These methods can also be used to assess somatic hypermutation (SHM) and to track minimal residual disease (MRD).

Malignant cells that remain in the bone marrow following treatment are a major cause of disease relapse. MRD testing by NGS offers enhanced sensitivity and specificity (compared to MRD testing by flow cytometry), and allows residual cells to be identified at very low levels and monitored throughout the different stages of disease.

Invivoscribe can provide you with the necessary tools to accommodate your needs. From gel detection to NGS, we can help you accurately identify and track hematologic biomarkers.

For additional information on the detection methods available and the biomarkers offered, please refer to the respective product sections of this catalog.

Immunoglobulin and T-Cell Receptor Gene Rearrangements and the Principle and Method of Clonality Testing

The adaptive vertebrate immune system produces a repertoire of immunoglobulin and T-cell receptor molecules using a relatively limited number of heritable germline gene segments. Somatic gene rearrangement is the fundamental mechanism used to generate different immunoglobulin and T-cell receptor molecules, each with unique binding specificity. Lymphocytes undergo gene rearrangements to assemble CDR3 coding regions that are unique in both size and DNA sequence. Since leukemias and lymphomas arise from the malignant transformation of a single cell, they share clonal rearrangement(s) of the antigen receptor genes. This is the basis for clonality testing[3].

References:

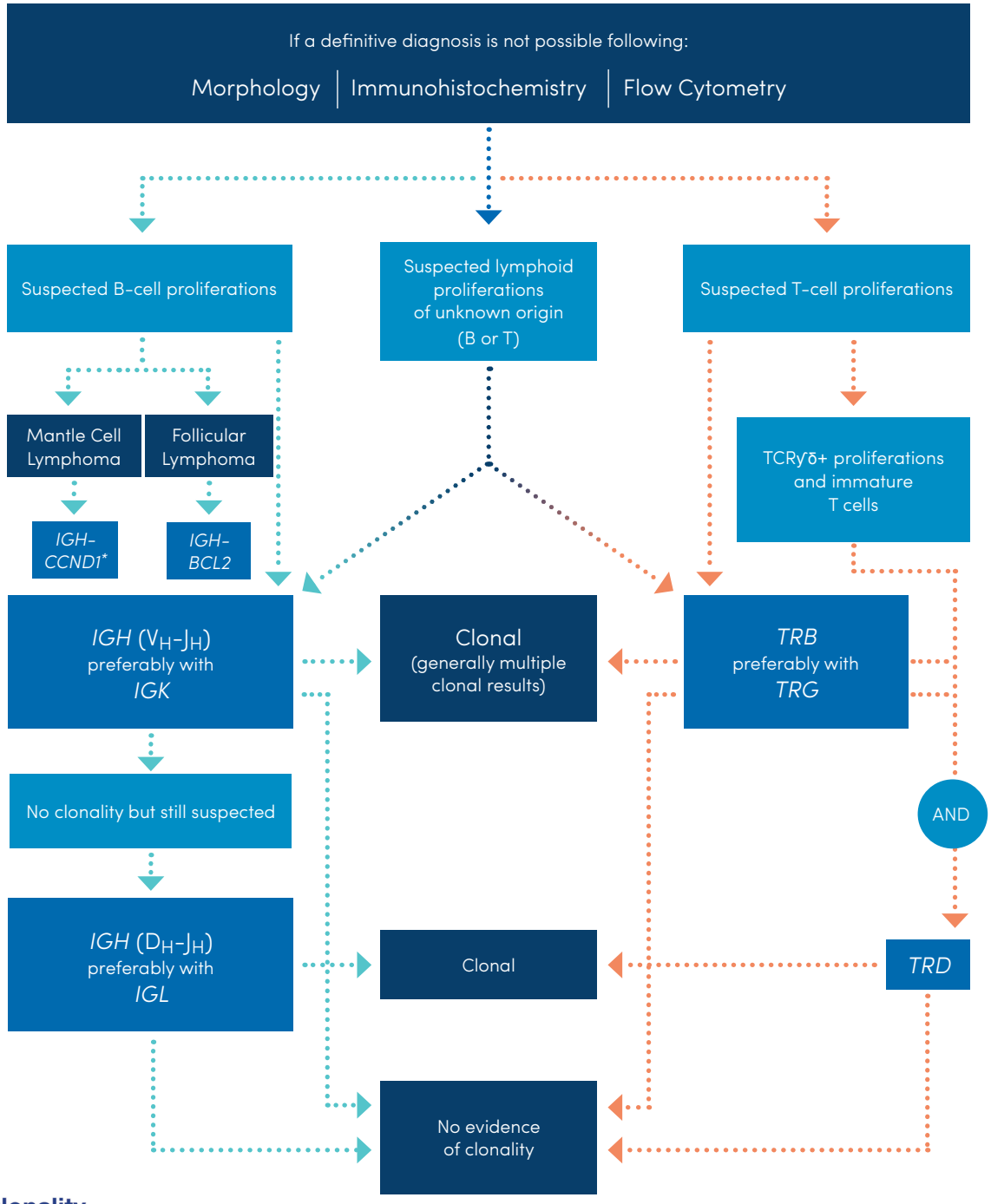
1. Tonegawa, S. Somatic Generation of Antibody Diversity. Nature 302:575-581 (1983)

2. Expression of T-cell receptor genes during early T-cell development. Immunol Cell Biol. 2008 Feb;86(2):166-74. Epub 2007 Oct 23.

3. Miller, J. E. (2013). Principle of Immunoglobulin and T cell Receptor Gene Rearrangement. In Cheng, L., Zhang, D., Eble, J. N. (Eds), *Molecular Genetic Pathology* (2nd Ed., Sections 30.2.7.13 and 30.2.7.18). pp825 – 856. New York, USA: Springer Science & Business Media.

Test Algorithm for Suspect Lymphoproliferations

Developed in concert with the EuroClonality/BIOMED-2 group for PCR-based clonality assessment of suspected B- and T-cell lymphoproliferative disorders.



EuroClonality

*Previously known as *BCL1*/JH

Results should be considered in the context of all available clinical, histological and immunophenotypic data.

Warranty and Liability

Invivoscribe Technologies, Inc. (Invivoscribe®) is committed to providing the highest quality products. Invivoscribe® warrants that the products meet or exceed the performance standards described in the Instructions For Use, as to products with such an insert. If a product is covered by product specifications and does not perform as specified, our policy is to replace the product or credit the full purchase price. No other warranties of any kind, expressed or implied, are provided by Invivoscribe Technologies. Invivoscribe Technologies liability shall not exceed the purchase price of the product. Invivoscribe shall have no liability for direct, indirect, consequential, or incidental damages arising from the use, results of use, or inability to use its products; product efficacy under purchaser controlled conditions in purchaser's laboratory must be established and continually monitored through purchaser defined and controlled processes including but not limited to testing of positive, negative, and blank controls every time a sample is tested. Ordering, acceptance, and use of product constitutes purchaser acceptance of sole responsibility for assuring product efficacy and purchaser agreement to the limitation of liability set forth in this paragraph.

NOTICE: These are *in vitro* diagnostic products and are not available for sale or use within North America. Many of the products listed in the section that follows may be covered by one or more of the following: United States Patent 7785783, and additional patents and patent applications pending in the United States and elsewhere. All of these patents and applications are licensed exclusively to Invivoscribe® worldwide (except Australia) across the field of hematopathology, including diagnostics, monitoring, prognosis and treatment. These products require nucleic acid amplification methods such as Polymerase Chain Reaction (PCR). No license under these patents to use amplification processes or enzymes is conveyed expressly or by implication to the purchaser by the purchase of these products.

Next-Generation Sequencing (NGS)

CE IVD Assays

LymphoTrack® Dx Assays

Determine clonality and track clonal populations with the same reagents and workflow.

LymphoTrack Dx Assay kits are designed for the identification of gene rearrangements in hematologic samples utilizing next-generation sequencing (NGS) technologies. These assays take advantage of the wealth and depth of data generated by the Illumina® MiSeq® and Thermo Fisher Scientific® Ion PGM™ platforms.

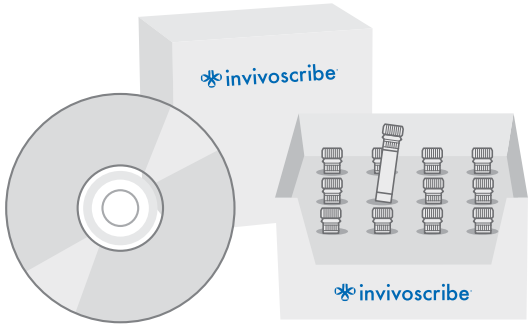
The Invivoscribe NGS assays offer significant improvements over conventional fragment analysis of B- and T-cell gene rearrangements by providing detailed information regarding the DNA sequences, sequence frequency, and mutational status (*IGHV* Leader and *IGH* FR1 only) of each clonotype.

LymphoTrack Dx Assay kits are a complete solution. Kits contain ready-to-use indexed amplification master mixes, necessary controls, and complimentary bioinformatics software. As primers are designed with barcoded indices and adapters, sequencing libraries can be generated with a one-step PCR, streamlining the overall workflow, eliminating the need for a post-PCR ligation step, and reducing the potential for sample cross contamination.

The per sample testing costs can be reduced by pooling samples from different LymphoTrack Dx Assays into a single sequencing run. The included bioinformatics software will sort the complex NGS data for easy analysis and visualization of individual samples.

Detailed instructions for use are provided with all kits and the Invivoscribe technical support team is always available to answer your questions.

For more information, please visit www.invivoscribe.com



Key Benefits

- One-step PCR for amplicon and library generation
- Identify, track, and assess mutation status of B- and T-cell gene rearrangements
- Sequence amplicons from any LymphoTrack Dx kit together
- Included bioinformatics software for easy analysis and interpretation

NOTICE:
The LymphoTrack Dx Assays are *in vitro* diagnostic products and are not available for sale or use within North America. For more information regarding the research use only reagents, please see the Next-Generation Sequencing - LymphoTrack section.

LymphoTrack® Dx

IGHV Leader Somatic Hypermutation Assay

LymphoTrack Dx *IGHV* Leader Somatic Hypermutation Assay

Intended Use

The LymphoTrack Dx *IGHV* Leader Somatic Hypermutation Assay for the Illumina® MiSeq® is an *in vitro* diagnostic product intended for next-generation sequencing (NGS) based determination of the frequency distribution of *IGH* gene rearrangements, as well as the degree of somatic hypermutation (SHM) of rearranged genes in patients suspected of having lymphoproliferative disease. This assay helps identify lymphoproliferative disorders, and it offers an aid in the identification of disease prognosis. If you would like to test for *IGHV* somatic hypermutation using the Thermo Fisher® Ion PGM™ platform, please refer to the LymphoTrack Dx *IGH* FR1 Assay (9-121-0007).

Summary and Explanation of the Test

The NGS LymphoTrack Dx *IGHV* Leader Somatic Hypermutation Assay for the Illumina® MiSeq® represents a significant improvement over clonality assays using fragment analysis as it efficiently detects the majority of *IGH* gene rearrangements using a single multiplex master mix, identifies the DNA sequence specific for each clonal gene rearrangement, and assesses the somatic hypermutation rate of clonal samples in the same workflow.

The single multiplex master mixes target the Leader (VHL) and the joining (JH) gene regions of the *IGH* locus and are designed with Illumina® adapters and indices (8 included in Kit A and 24 included in the Panel). This allows for a one-step PCR reaction and pooling of amplicons from several different samples and targets into a single Illumina® MiSeq® run. No post-PCR ligation step is required.

The included LymphoTrack Dx Bioinformatics Software enables simplified analysis and visualization of individual sample data.

Positive (clonal positive, SHM negative), negative (polyclonal) and SHM (clonal positive, SHM positive) DNA controls are included in the kits.

Background

The human immunoglobulin heavy chain (*IGH*) gene locus on chromosome 14 (14q32.3) includes 46-52 functional and 30 nonfunctional variable (VH) gene segments, 27 functional diversity (DH) gene segments, and 6 functional joining (JH) gene segments spread over 1,250 kilobases.

During B-cell development, genes encoding the IGH protein are assembled from multiple polymorphic gene segments that undergo rearrangements and selection, generating cell specific VH-DH-JH combinations that are unique in both length and sequence.

Since leukemias and lymphomas originate from the malignant transformation of individual lymphoid cells, all leukemias and lymphomas generally share one or more cell-specific or "clonal" antigen receptor gene rearrangements. Therefore, tests that detect *IGH* clonal rearrangements can be useful in the study of B- cell malignancies. An additional level of diversity is further generated in the antigen receptors by introducing point mutations in the variable regions, also named SHM. In instances where there is a high degree of SHM, there is the risk that primers located within the variable region will not be able to bind and clonal products will not amplify. In these cases, the leader primers located upstream of the variable region can be beneficial for the detection of clonal products, due to the conserved nature of the VHL region. In addition, the SHM rate of the entire variable gene can be determined using the VHL primers.

Determining the immunoglobulin variable heavy chain gene (*IGHV*) hypermutation rate is considered a gold standard for determining the prognosis of patients with chronic lymphocytic leukemia (CLL) and small lymphocytic lymphoma (SLL). In addition, NGS methods can improve disease stratification.

Specimen Requirement

50 ng of high-quality genomic DNA.

Reference

1. B Stamatopoulous et al., *Leukemia* 4:837-845 (2017).
2. F Davi et al., *Leukemia* 22: 212-214 (2008).
3. JE Miller et al., *Molecular Genetic Pathology* (2nd ed.). Springer Science & Business Media. 2013: 302.2.7.13 and 30.2.7.18.
4. P Ghia et al., *Leukemia* 21: 2-3 (2007).
5. P Ghia et al., *Blood* 105: 1678-1685 (2005).
6. S Tonegawa. *Nature* 302: 575-581 (1983).

Simplified representation of the immunoglobulin heavy chain (*IGH*) gene locus on chromosome 14. Depicted are the variable (VH) and downstream consensus joining (JH) region genes involved in rearrangements. Upstream of the variable gene segments, the leader sequence (VHL) is also depicted. Diversity region genes are not depicted.

Reagents - MiSeq® Detection

Kit A Components

Master Mix Name	Index #
IGH Leader MiSeq 01	A001
IGH Leader MiSeq 02	A002
IGH Leader MiSeq 03	A003
IGH Leader MiSeq 04	A004
IGH Leader MiSeq 05	A005
IGH Leader MiSeq 06	A006
IGH Leader MiSeq 07	A007
IGH Leader MiSeq 08	A008

Controls
IGH SHM POS (+) Qty. 1
IGH POS (+) Qty. 1
NGS NEG (-) Qty. 1

Panel Components (includes all master mixes from Kit A plus the items below)

Master Mix Name	Index #	Master Mix Name	Index #
IGH Leader MiSeq 09	A009	*IGH* Leader MiSeq 18	A018
IGH Leader MiSeq 10	A010	*IGH* Leader MiSeq 19	A019
IGH Leader MiSeq 11	A011	*IGH* Leader MiSeq 20	A020
IGH Leader MiSeq 12	A012	*IGH* Leader MiSeq 21	A021
IGH Leader MiSeq 13	A013	*IGH* Leader MiSeq 22	A022
IGH Leader MiSeq 14	A014	*IGH* Leader MiSeq 23	A023
IGH Leader MiSeq 15	A015	*IGH* Leader MiSeq 25	A025
IGH Leader MiSeq 16	A016	*IGH* Leader MiSeq 27	A027

Controls
IGH SHM POS (+) Qty. 3
IGH POS (+) Qty. 3
NGS NEG (-) Qty. 3

Rank	Sequence	Length	Merge count	V-gene	J-gene	% Total reads	Cumulative %	Mutation rate partial V-gene (%)	In-frame (Y/N)	No stop codon (Y/N)	V-coverage
1	TTCTCGTGGTG	455	29603	IGHV4-59_08	IGHJ4_02	9.93	9.93	11.26	Y	Y	98.63
2	CTCGCCCTCCT	463	205	IGHV5-51_01	IGHJ4_02	0.07	9.99	0.00	Y	Y	99.66
3	GGTTTTCCTTG	484	201	IGHV3-7_01	IGHJ4_02	0.07	10.06	7.77	Y	Y	100.00
4	CTCGCCCTCCT	463	185	IGHV5-51_01	IGHJ5_02	0.06	10.12	6.08	Y	Y	99.32
5	CTCGCCCTCCT	469	170	IGHV5-51_01	IGHJ4_02	0.06	10.18	0.00	Y	Y	99.32
6	CTCGCCCTCCT	466	160	IGHV5-51_01	IGHJ4_02	0.05	10.23	0.00	Y	Y	99.66
7	CTGCTGCTGAC	460	159	IGHV2-5_10	IGHJ5_02	0.05	10.29	8.08	Y	Y	97.64
8	GGTTTTCCTTG	493	156	IGHV3-48_02	IGHJ6_02	0.05	10.34	3.72	Y	Y	98.99
9	CTCGCCCTCCT	334	153	IGHV5-51_02	IGHJ2_01	0.05	10.39	3.72	Y	N	27.70
10	CTCGCCCTCCT	334	152	IGHV5-51_02	IGHJ2_01	0.05	10.44	3.38	Y	N	26.01

Example Data. The top 10 sequences from a read summary generated by the LymphoTrack Dx Software - MiSeq® with the SHM mutation rate and predictions pertaining to whether a sequence is in-frame or contains a premature stop codon are depicted. To learn more about the LymphoTrack Dx software, please refer to the LymphoTrack Dx Bioinformatics Software section.

Ordering Information

Catalog #	Products	Quantity Components
9-121-0059	LymphoTrack® Dx *IGHV* Leader Somatic Hypermutation Assay Kit A - MiSeq®	8 indices - 5 sequencing reactions each
9-121-0069	LymphoTrack® Dx *IGHV* Leader Somatic Hypermutation Assay Panel - MiSeq®	24 indices - 5 sequencing reactions each
9-500-0009	LymphoTrack® Dx Software - MiSeq®	1 CD complimentary with purchase

CE IVD These products are CE-IVD assays for *in vitro* diagnostic use.

LymphoTrack® Dx
IGH Assays

LymphoTrack Dx *IGH* FR1/2/3 Assays

Intended Uses

LymphoTrack Dx *IGH* FR1 Assays
The LymphoTrack Dx *IGH* FR1 Assays for the Illumina® MiSeq® or Thermo Fisher Scientific® Ion PGM™ are *in vitro* diagnostic products intended for next-generation sequencing (NGS) based determination of the frequency distribution of *IGH* gene rearrangements as well as the degree of somatic hypermutation (SHM) within Framework Region 1 (FR1) of the *IGH* locus, of rearranged genes in patients suspected of having lymphoproliferative disease. These assays help identify lymphoproliferative disorders, and they offer an aid in the identification of disease prognosis.

LymphoTrack Dx *IGH* FR2 Assays
The LymphoTrack Dx *IGH* FR2 Assays are *in vitro* diagnostic products intended for NGS analysis for the Illumina® MiSeq® or Thermo Fisher Scientific Ion PGM instrument. The assays will determine the frequency distribution of VH-JH gene rearrangements within Framework Region 2 (FR2) of the *IGH* locus in patients suspected of having lymphoproliferative disease. These assays aid in the identification of lymphoproliferative disorders.

LymphoTrack Dx *IGH* FR3 Assays
The LymphoTrack Dx *IGH* FR3 Assays are *in vitro* diagnostic products intended for NGS analysis for the Illumina® MiSeq® or Thermo Fisher Scientific Ion PGM instrument. The assays will determine the frequency distribution of VH-JH gene rearrangements within Framework Region 3 (FR3) of the *IGH* locus in patients suspected of having lymphoproliferative disease. These assays aid in the identification of lymphoproliferative disorders.

Summary and Explanation of the Test

The LymphoTrack Dx *IGH* Assays represent a significant improvement over conventional clonality assessment methods utilizing fragment analysis by providing three important and complementary uses in a single workflow:

1. Detection of clonal populations.
2. Identification of sequence information required to track clonal rearrangements in subsequent samples.
3. The LymphoTrack Dx *IGH* framework 1 FR1 master mixes provide the degree of SHM in the immunoglobulin variable heavy chain (*IGHV*) gene locus.

These assays utilize a single multiplex master mix to target each conserved *IGH* Framework Region (FR1, FR2, and FR3) within the VH and the JH regions described in lymphoid malignancies. The included primers are designed with Illumina® or Thermo Fisher Scientific adapters (8-24 and 12, respectively). This allows up to 24 samples on MiSeq® and 12 samples on Ion PGM™ to be sequenced at the same time with any of the individual FRs.

In addition, amplicons generated with different FR master mixes or Invivoscribe LymphoTrack Dx kits (such as *IGK* or *TRG*) can be pooled together in the same sequencing library to reduce testing costs. The associated LymphoTrack Dx Software provides interpretation of the data via a simple and streamlined method of analysis and visualization. By following the guidelines provided in the instructions for use, samples can be interpreted for evidence of clonality and SHM.

Positive clonal (SHM negative) and negative polyclonal DNA controls are included in kits. A clonal SHM positive control can be purchased separately (cat#: 4-088-0008).

Background

The human immunoglobulin heavy chain (*IGH*) gene locus on chromosome 14 (14q32.3) includes 46-52 functional and 30 non-functional variable (VH), 27 functional diversity (DH), and 6 functional joining (JH) gene segments. The VH gene segments can be further broken down into three conserved frameworks (FR) and two variable complementarity-determining regions (CDRs).

During development of lymphoid cells, antigen receptor genes undergo somatic gene rearrangements. Specifically during B-cell development, IGH molecules are assembled from multiple polymorphic gene segments that undergo rearrangements generating VH-DH-JH combinations unique in both length and sequence. Since leukemias and lymphomas originate from the malignant transformation of individual lymphoid cells, all leukemias and lymphomas generally share one or more cell-specific or "clonal" antigen receptor gene rearrangements.

In addition, the *IGHV* hypermutation status obtained with the LymphoTrack Dx *IGH* FR1 master mixes, provides important prognostic information for patients with chronic lymphocytic leukemia (CLL) and small lymphocytic lymphoma (SLL). The SHM rate has been shown to have clinical relevance, as there is a clear distinction in the median survival of patients with and without SHM.

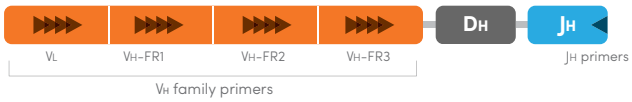
Specimen Requirement

50 ng of high-quality genomic DNA.

Reference

1. S Tonegawa. *Nature* 302: 575-581 (1983).
2. JE Miller et al., *Molecular Genetic Pathology* (2nd ed.). Springer Science & Business Media. 2013: 30.2.7.13 and 30.2.7.18.
3. KJ Trainor et al., *Blood* 75: 2220-2222 (1990).
4. P Ghia. *Leukemia* 21: 1-3(2007).

Simplified Representation of the *IGH* Gene



Simplified depiction of variable (VH) and downstream consensus joining (JH) region genes involved in gene rearrangements.

Reagents - MiSeq® Detection

The LymphoTrack Dx *IGH* FR1/2/3 Assays contain components from respective individual FR kit A's or panels.

LymphoTrack Dx *IGH* FR1 Components			LymphoTrack Dx *IGH* FR2 Components			LymphoTrack Dx *IGH* FR3 Components		
Master Mix Name	Index #		Master Mix Name	Index #		Master Mix Name	Index #	
IGH FR1 MiSeq 01	A001	KIT A	*IGH* FR2 MiSeq 01	A001	KIT A	*IGH* FR3 MiSeq 01	A001	KIT A
IGH FR1 MiSeq 02	A002		*IGH* FR2 MiSeq 02	A002		*IGH* FR3 MiSeq 02	A002	
IGH FR1 MiSeq 03	A003		*IGH* FR2 MiSeq 03	A003		*IGH* FR3 MiSeq 03	A003	
IGH FR1 MiSeq 04	A004		*IGH* FR2 MiSeq 04	A004		*IGH* FR3 MiSeq 04	A004	
IGH FR1 MiSeq 05	A005		*IGH* FR2 MiSeq 05	A005		*IGH* FR3 MiSeq 05	A005	
IGH FR1 MiSeq 06	A006		*IGH* FR2 MiSeq 06	A006		*IGH* FR3 MiSeq 06	A006	
IGH FR1 MiSeq 07	A007		*IGH* FR2 MiSeq 07	A007		*IGH* FR3 MiSeq 07	A007	
IGH FR1 MiSeq 08	A008		*IGH* FR2 MiSeq 08	A008		*IGH* FR3 MiSeq 08	A008	
IGH FR1 MiSeq 09	A009		*IGH* FR2 MiSeq 09	A009		*IGH* FR3 MiSeq 09	A009	
IGH FR1 MiSeq 10	A010		*IGH* FR2 MiSeq 10	A010		*IGH* FR3 MiSeq 10	A010	
IGH FR1 MiSeq 11	A011		*IGH* FR2 MiSeq 11	A011		*IGH* FR3 MiSeq 11	A011	
IGH FR1 MiSeq 12	A012		*IGH* FR2 MiSeq 12	A012		*IGH* FR3 MiSeq 12	A012	
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IGH FR1 MiSeq 22	A022		*IGH* FR2 MiSeq 22	A022		*IGH* FR3 MiSeq 22	A022	
IGH FR1 MiSeq 23	A023		*IGH* FR2 MiSeq 23	A023		*IGH* FR3 MiSeq 23	A023	
IGH FR1 MiSeq 25	A025		*IGH* FR2 MiSeq 25	A025		*IGH* FR3 MiSeq 25	A025	
IGH FR1 MiSeq 27	A027		*IGH* FR2 MiSeq 27	A027		*IGH* FR3 MiSeq 27	A027	

Kit A's contain indices *IGH* FRX A001 to A008. Panels contain all master mixes listed above.

Controls in Individual FR (1, 2, or 3) Kit A's	Controls in Individual FR (1, 2, or 3) Panels	Controls in Combo FR 1/2/3 Kit A	Controls in Combo FR 1/2/3 Panel
IGH POS (+) Qty. 1	*IGH* POS (+) Qty 3	*IGH* POS (+) Qty. 2	*IGH* POS (+) Qty. 6
NGS NEG (-) Qty. 1	NGS NEG (-) Qty 3	NGS NEG (-) Qty. 2	NGS NEG (-) Qty. 6

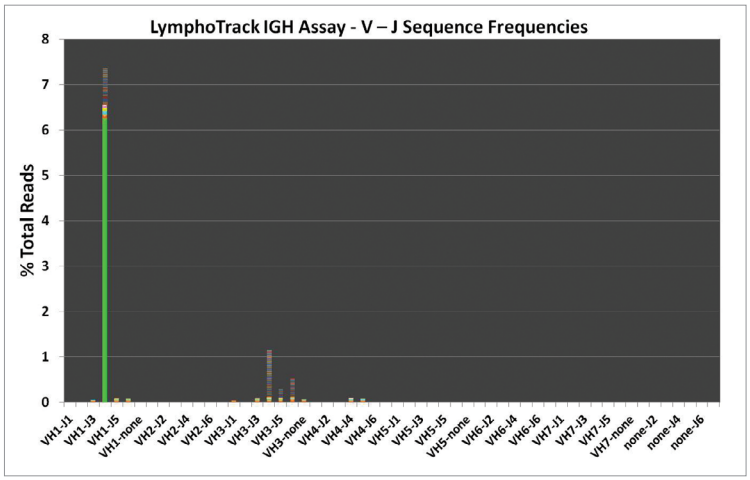
LymphoTrack Dx *IGH* FR1/2/3 Assays cont.

Reagents – Ion PGM™ Detection

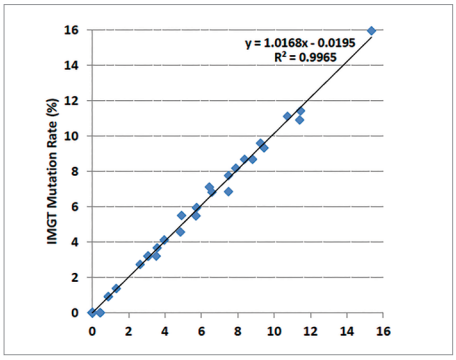
The LymphoTrack Dx *IGH* FR1/2/3 Assays contain components from respective individual FR Assays.

LymphoTrack Dx *IGH* FR1 Components		LymphoTrack Dx *IGH* FR2 Components		LymphoTrack Dx *IGH* FR3 Components	
Master Mix Name	Index #	Master Mix Name	Index #	Master Mix Name	Index #
IGH FR1 PGM 01	IonXpress_001	*IGH* FR2 PGM 01	IonXpress_001	*IGH* FR3 PGM 01	IonXpress_001
IGH FR1 PGM 02	IonXpress_002	*IGH* FR2 PGM 02	IonXpress_002	*IGH* FR3 PGM 02	IonXpress_002
IGH FR1 PGM 03	IonXpress_003	*IGH* FR2 PGM 03	IonXpress_003	*IGH* FR3 PGM 03	IonXpress_003
IGH FR1 PGM 04	IonXpress_004	*IGH* FR2 PGM 04	IonXpress_004	*IGH* FR3 PGM 04	IonXpress_004
IGH FR1 PGM 07	IonXpress_007	*IGH* FR2 PGM 07	IonXpress_007	*IGH* FR3 PGM 07	IonXpress_007
IGH FR1 PGM 08	IonXpress_008	*IGH* FR2 PGM 08	IonXpress_008	*IGH* FR3 PGM 08	IonXpress_008
IGH FR1 PGM 09	IonXpress_009	*IGH* FR2 PGM 09	IonXpress_009	*IGH* FR3 PGM 09	IonXpress_009
IGH FR1 PGM 10	IonXpress_010	*IGH* FR2 PGM 10	IonXpress_010	*IGH* FR3 PGM 10	IonXpress_010
IGH FR1 PGM 11	IonXpress_011	*IGH* FR2 PGM 11	IonXpress_011	*IGH* FR3 PGM 11	IonXpress_011
IGH FR1 PGM 12	IonXpress_012	*IGH* FR2 PGM 12	IonXpress_012	*IGH* FR3 PGM 12	IonXpress_012
IGH FR1 PGM 13	IonXpress_013	*IGH* FR2 PGM 13	IonXpress_013	*IGH* FR3 PGM 13	IonXpress_013
IGH FR1 PGM 14	IonXpress_014	*IGH* FR2 PGM 14	IonXpress_014	*IGH* FR3 PGM 14	IonXpress_014

Controls in Individual FR (1,2, or 3) Kits	Controls in FR 1/2/3 Kit
IGH POS (+) Qty. 2	*IGH* POS (+) Qty. 4
NGS NEG (-) Qty. 2	NGS NEG (-) Qty. 4



V-J Sequence Frequencies. The LymphoTrack Dx Software provides a stacked bar graph depicting the relative frequencies of the 200 most prevalent VH–JH rearrangements identified in a sample. To learn more about the LymphoTrack Dx software, please refer to the LymphoTrack Dx Bioinformatics Software section.



Comparison of SHM Analysis Methods. The SHM rate of 51 CLL samples was determined by the LymphoTrack Dx *IGH* FR1 Assay – MiSeq® and analyzed with both the LymphoTrack Dx Software – MiSeq® and IMGT analysis.

Ordering Information

Catalog #	Products	Quantity Components
9-121-0129	LymphoTrack® Dx *IGH* FR1/2/3 Assay Kit A - MiSeq®	8 + 8 + 8 indices - 5 sequencing reactions each
9-121-0139	LymphoTrack® Dx *IGH* FR1/2/3 Assay Panel - MiSeq®	24 + 24 + 24 indices - 5 sequencing reactions each
9-121-0009	LymphoTrack® Dx *IGH* FR1 Assay Kit A - MiSeq®	8 indices - 5 sequencing reactions each
9-121-0039	LymphoTrack® Dx *IGH* FR1 Assay Panel - MiSeq®	24 indices - 5 sequencing reactions each
9-121-0089	LymphoTrack® Dx *IGH* FR2 Assay Kit A - MiSeq®	8 indices - 5 sequencing reactions each
9-121-0099	LymphoTrack® Dx *IGH* FR2 Assay Panel - MiSeq®	24 indices - 5 sequencing reactions each
9-121-0109	LymphoTrack® Dx *IGH* FR3 Assay Kit A - MiSeq®	8 indices - 5 sequencing reactions each
9-121-0119	LymphoTrack® Dx *IGH* FR3 Assay Panel - MiSeq®	24 indices - 5 sequencing reactions each
9-500-0009	LymphoTrack® Dx Software - MiSeq®	1 CD complimentary with purchase
9-121-0057	LymphoTrack® Dx *IGH* FR1/2/3 Assay - PGM™	12 + 12 + 12 indices - 5 sequencing reactions each
9-121-0007	LymphoTrack® Dx *IGH* FR1 Assay - PGM™	12 indices - 5 sequencing reactions each
9-121-0037	LymphoTrack® Dx *IGH* FR2 Assay - PGM™	12 indices - 5 sequencing reactions each
9-121-0047	LymphoTrack® Dx *IGH* FR3 Assay - PGM™	12 indices - 5 sequencing reactions each
9-500-0007	LymphoTrack® Dx Software - PGM™	1 CD complimentary with purchase

CE IVD These products are CE-IVD assays for *in vitro* diagnostic use.

LymphoTrack® Dx
IGK Assays

LymphoTrack Dx *IGK* Assays

Intended Use

The LymphoTrack Dx *IGK* Assays for the Illumina® MiSeq® or Thermo Fisher Scientific® Ion PGM™ are *in vitro* diagnostic products intended for next-generation sequencing (NGS) based determination of the frequency distribution of *IGK* gene rearrangements in patients suspected of having lymphoproliferative disease. These assays aid in the identification of lymphoproliferative disorders.

Summary and Explanation of the Test

In contrast to the IdentiClone™ fragment analysis assays for clonality that utilize two master mixes, these NGS assays contain a single multiplex master mix to target conserved regions of the *IGK* gene locus described in lymphoid malignancies, thereby, reducing sample DNA requirements and simplifying the testing workflow. The LymphoTrack Dx *IGK* master mix primers are also designed with Illumina® or Thermo Fisher Scientific adapters and up to 24 different indices. This allows amplicons generated from different indexed *IGK* master mixes to be pooled into a single library for loading onto one MiSeq® flow cell or Ion PGM sequencing chip.

The associated LymphoTrack Dx Software is capable of sorting complex NGS data by gene target, providing users the ability to reduce per sample testing costs by sequencing amplicons from any LymphoTrack Dx Assay (*e.g. IGH, IGK, TRB, TRG*) at the same time. In addition, the LymphoTrack Dx Software provides an easy and streamlined method for visualization of data and guidelines provided in the instructions for use allow samples to be interpreted for evidence or no evidence of clonality.

Positive clonal and negative polyclonal DNA controls are included in kits.

Background

The LymphoTrack Dx *IGK* Assays represent a significant improvement over existing fragment analysis clonality assays by providing two important and complementary uses:

1. Detection of initial clonal populations.
2. Identification of sequence information required to track clonal rearrangements in subsequent samples.

The human immunoglobulin kappa (*IGK*) gene locus on chromosome 2 (2p11.2) includes 76 V (variable) genes spanning 7 subgroups and 5 J (joining) gene segments upstream of the Cκ region.

During lymphoid cell development, antigen receptor genes undergo somatic gene rearrangements. Specifically, during B-cell development, genes encoding IGK molecules are assembled from multiple polymorphic gene segments that generate V-J combinations unique in both length and sequence.

In addition, the kappa deleting element (Kde), approximately 24 kb downstream of the Jκ-Cκ region can also rearrange with Vκ gene segments and the isolated recombination signal sequence in the Jκ-Cκ intron (Jκ-Cκ INTR).

Since leukemias and lymphomas originate from the malignant transformation of individual lymphoid cells, all leukemias and lymphomas generally share one or more cell-specific or "clonal" antigen receptor gene rearrangements. Therefore, tests that detect *IGK* clonal rearrangements can be useful in the study of B- and T-cell malignancies.

Specimen Requirement

50 ng of high-quality genomic DNA.

Reference

1. EP Rock et al., J *Exp Med* 179 (1): 323-8 (1994).
2. JE Miller et al., *Molecular Diagnostic* 4 (2): 102-117(1999).

Simplified Representation of the *IGK* Gene

Depicted are the variable region (Vκ) or variable intragenic Jκ-Cκ intron (Jκ-Cκ INTR) genes involved in *IGK* gene rearrangements in addition to the downstream consensus joining region genes (Jκ) or kappa deleting element (Kde).

Reagents - MiSeq® Detection

Kit A Components

Master Mix Name	Index #
IGK MiSeq 01	A001
IGK MiSeq 02	A002
IGK MiSeq 03	A003
IGK MiSeq 04	A004
IGK MiSeq 05	A005
IGK MiSeq 06	A006
IGK MiSeq 07	A007
IGK MiSeq 08	A008

Controls	
IGK POS (+) Qty. 1	NGS NEG (-) Qty. 1

Panel Components (includes all master mixes from Kit A plus the items below)

Master Mix Name	Index #	Master Mix Name	Index #
IGK MiSeq 09	A009	*IGK* MiSeq 18	A018
IGK MiSeq 10	A010	*IGK* MiSeq 19	A019
IGK MiSeq 11	A011	*IGK* MiSeq 20	A020
IGK MiSeq 12	A012	*IGK* MiSeq 21	A021
IGK MiSeq 13	A013	*IGK* MiSeq 22	A022
IGK MiSeq 14	A014	*IGK* MiSeq 23	A023
IGK MiSeq 15	A015	*IGK* MiSeq 25	A025
IGK MiSeq 16	A016	*IGK* MiSeq 27	A027

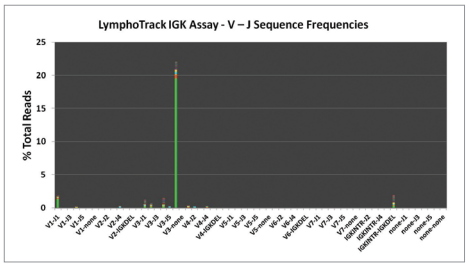
Controls	
IGK POS (+) Qty. 3	NGS NEG (-) Qty. 3

Reagents - Ion PGM™ Detection

Assay Components

Master Mix Name	Index #	Master Mix Name	Index #
IGK PGM 01	IonXpress_001	*IGK* PGM 11	IonXpress_011
IGK PGM 02	IonXpress_002	*IGK* PGM 12	IonXpress_012
IGK PGM 04	IonXpress_004	*IGK* PGM 13	IonXpress_013
IGK PGM 08	IonXpress_008	*IGK* PGM 14	IonXpress_014
IGK PGM 09	IonXpress_009	*IGK* PGM 16	IonXpress_016
IGK PGM 010	IonXpress_010	*IGK* PGM 17	IonXpress_017

Controls	
IGK POS (+) Qty. 2	NGS NEG (-) Qty. 2



V-J Sequence Frequencies. The LymphoTrack Dx Software provides a stacked bar graph depicting the relative frequencies for the most prevalent rearrangements identified in a sample. To learn more about the LymphoTrack Dx software, please refer to the LymphoTrack Dx Bioinformatics Software section.

Ordering Information

Catalog #	Products	Quantity Components
9-122-0009	LymphoTrack® Dx *IGK* Assay Kit A - MiSeq®	8 indices - 5 sequencing reactions each
9-122-0019	LymphoTrack® Dx *IGK* Assay Panel - MiSeq®	24 indices - 5 sequencing reactions each
9-500-0009	LymphoTrack® Dx Software - MiSeq®	1 CD complimentary with purchase
9-122-0007	LymphoTrack® Dx *IGK* Assay - PGM™	12 indices - 5 sequencing reactions each
9-500-0007	LymphoTrack® Dx Software - PGM™	1 CD complimentary with purchase

CE IVD These products are CE-IVD assays for *in vitro* diagnostic use.

LymphoTrack® Dx
TRG Assay

LymphoTrack Dx *TRG* Assay

Intended Use

The LymphoTrack Dx *TRG* Assays for the Illumina® MiSeq® or Thermo Fisher Scientific® Ion PGM™ are *in vitro* diagnostic products intended for next-generation sequencing (NGS) based determination of the frequency distribution of *TRG* gene rearrangements in patients suspected with having lymphoproliferative disease. These assays aid in the identification of lymphoproliferative disorders.

Summary and Explanation of the Test

These assays utilize a single multiplex master mix to target conserved V and J regions of the human T-cell receptor gamma (*TRG*) that are described in lymphoid malignancies. Primers are designed with Illumina® or Thermo Fisher Scientific adapters and up to 24 different indices; thereby allowing amplicons generated from different *TRG* master mixes to be pooled together for sequencing on a single MiSeq® flow cell or Ion PGM chip.

The associated LymphoTrack Dx Software sorts complex NGS data by gene target, providing users the ability to reduce per sample testing costs by sequencing amplicons generated with any LymphoTrack Dx Assay (*e.g. IGH, IGK, TRB, TRG*) at the same time. In addition, the LymphoTrack Dx Software provides an easy and streamlined method for data visualization and guidelines provided in the instructions for use allow samples to be interpreted for evidence or no evidence of clonality.

Positive clonal and negative polyclonal DNA controls are included in kits.

Background

The LymphoTrack Dx *TRG* Assays represent a significant improvement over existing fragment analysis clonality assays by providing two important and complementary uses:

1. Detection of initial clonal populations.
2. Identification of sequence information required to track clonal rearrangements in subsequent samples.

The *TRG* gene locus on chromosome 7 (7q14) includes 14 V (variable region) genes (Group I, II, III, and IV), 5 J (joining region) gene segments, and 2 C (constant region) genes spread over 200 kilobases.

During development of lymphoid cells, antigen receptor genes undergo somatic gene rearrangements. Specifically during T-cell development, genes encoding TRG molecules are assembled from multiple polymorphic gene segments that undergo rearrangement generating V-J combinations unique in both length and sequence. Since leukemias and lymphomas originate from the malignant transformation of individual lymphoid cells, all leukemias and lymphomas generally share one or more cell-specific or "clonal" antigen receptor gene rearrangement. Therefore, tests that detect *TRG* clonal rearrangements can be useful in the study of B- and T-cell malignancies.

Specimen Requirement

50 ng of high-quality genomic DNA.

Reference

1. S Tonegawa. *Nature* 302: 575-581 (1983).
2. JE Miller et al., *Molecular Genetic Pathology* (2nd ed.). Springer Science & Business Media. 2013: 30.2.7.13.

Simplified Representation of the *TRG* Gene

Depicted are the variable region (V) genes and downstream consensus joining region genes (J) that are involved in *TRG* gene rearrangements.

Reagents - MiSeq® Detection

Kit A Components

Master Mix Name	Index #
TRG MiSeq 01	A001
TRG MiSeq 02	A002
TRG MiSeq 03	A003
TRG MiSeq04	A004
TRG MiSeq 05	A005
TRG MiSeq 06	A006
TRG MiSeq 07	A007
TRG MiSeq 08	A008
Controls	
TRG POS (+) Qty. 1	NGS NEG (-) Qty. 1

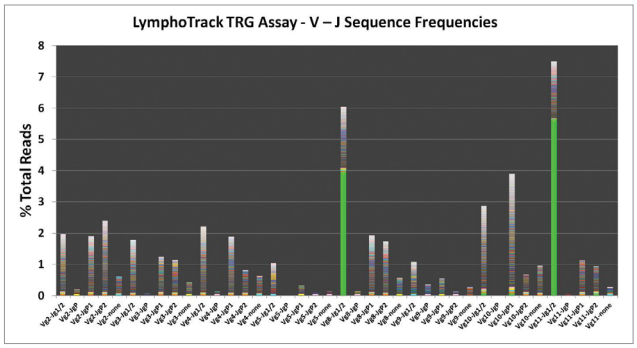
Panel Components (includes all master mixes from Kit A plus the items below)

Master Mix Name	Index #	Master Mix Name	Index #
TRG MiSeq 09	A009	*TRG* MiSeq 18	A018
TRG MiSeq 10	A010	*TRG* MiSeq 19	A019
TRG MiSeq 11	A011	*TRG* MiSeq 20	A020
TRG MiSeq 12	A012	*TRG* MiSeq 21	A021
TRG MiSeq 13	A013	*TRG* MiSeq 22	A022
TRG MiSeq 14	A014	*TRG* MiSeq 23	A023
TRG MiSeq 15	A015	*TRG* MiSeq 25	A025
TRG MiSeq 16	A016	*TRG* MiSeq 27	A027
Controls			
TRG POS (+) Qty. 3		NGS NEG (-) Qty. 3	

Reagents - Ion PGM™ Detection

Assay components

Master Mix Name	Index #	Master Mix Name	Index #	Master Mix Name	Index #
TRG PGM 01	IonXpress_001	*TRG* PGM 07	IonXpress_007	*TRG* PGM 11	IonXpress_011
TRG PGM 02	IonXpress_002	*TRG* PGM 08	IonXpress_008	*TRG* PGM 12	IonXpress_012
TRG PGM 03	IonXpress_003	*TRG* PGM 09	IonXpress_009	*TRG* PGM 13	IonXpress_013
TRG PGM 04	IonXpress_004	*TRG* PGM 10	IonXpress_010	*TRG* PGM 14	IonXpress_014
Controls					
TRG POS (+) Qty. 2		NGS NEG (-) Qty. 2			



V-J Sequence Frequencies. The LymphoTrack Dx bioinformatics software provides a stacked bar graph depicting the relative frequencies of the V-J rearrangements identified in the sample. To learn more about the LymphoTrack Dx software, please refer to the LymphoTrack Dx Bioinformatics Software section.

Ordering Information

Catalog #	Products	Quantity Components
9-227-0019	LymphoTrack® Dx *TRG* Assay Kit A - MiSeq®	8 indices - 5 sequencing reactions each
9-227-0009	LymphoTrack® Dx *TRG* Assay Panel - MiSeq®	24 indices - 5 sequencing reactions each
9-500-0009	LymphoTrack® Dx Software - MiSeq®	1 CD complimentary with purchase
9-227-0007	LymphoTrack® Dx *TRG* Assay - PGM™	12 indices - 5 sequencing reactions each
9-500-0007	LymphoTrack® Dx Software - PGM™	1 CD complimentary with purchase

CE IVD These products are CE-IVD assays for *in vitro* diagnostic use.

LymphoTrack® Dx
TRB Assays

LymphoTrack Dx *TRB* Assays

Intended Use

The LymphoTrack® Dx *TRB* Assay is an *in vitro* diagnostic product intended for next-generation sequencing (NGS) for the Illumina MiSeq® instrument. The assay will determine the frequency distribution of *TRB* Vβ-Jβ gene rearrangements in patients suspected of having lymphoproliferative disease. This assay aids in the identification of lymphoproliferative disorders.

Summary and Explanation of the Test

In contrast to the IdentiClone™ fragment analysis assays for clonality that utilizes three master mixes, this NGS-based assay contains a single multiplex master mix to target conserved V and J regions of the *TRB* gene locus described in lymphoid malignancies, thereby, reducing sample DNA requirements and simplifying the testing workflow. The LymphoTrack Dx *TRB* master mix primers are designed with Illumina® adapters and 8 (Kit A) or 24 (Panel) different indices. This allows amplicons generated from different indexed *TRB* master mixes to be pooled into a single library for loading onto one MiSeq® flow cell.

The associated LymphoTrack Dx Software is capable of sorting complex NGS data by gene target, offering a second layer of multiplexing. This provides users the ability to reduce per sample testing costs by sequencing amplicons from any LymphoTrack Dx MiSeq® Assay (e.g. *TRB, TRG, IGH, IGK*) at the same time. In addition, the LymphoTrack Dx Software provides an easy and streamlined method for visualization of data. Guidelines to interpret samples for evidence or no evidence of clonality are included in the instructions for use provided with each kit.

Positive clonal and negative polyclonal DNA controls are included in kits.

Background

The LymphoTrack Dx *TRB* Assays represent a significant improvement over fragment analysis methods for clonality testing by providing two important and complementary uses:

1. Detection of initial clonal populations.
2. Identification of sequence information required to track clonal rearrangements in subsequent samples.

The human T cell receptor beta (*TRB*) gene locus on chromosome 7 (7q34) includes 65 Vβ (variable) gene segments, followed by two separate clusters of genes each containing a Dβ (diversity) gene, several Jβ (joining) genes, and a Cβ (constant) region spread over 685 kilobases. The 2 Cβ genes, *TRBC1* and *TRBC2*, encode highly homologous products with no functional difference.

During lymphoid cell development, antigen receptor genes undergo somatic gene rearrangements. Specifically, during T-cell development genes encoding TRB molecules are assembled from multiple polymorphic gene segments that generate Vβ - Dβ - Jβ combinations unique in both length and sequence.

Since leukemias and lymphomas originate from the malignant transformation of individual lymphoid cells, all leukemias and lymphomas generally share one or more cell-specific or "clonal" antigen receptor gene rearrangements. Therefore, tests that detect *TRB* clonal rearrangements can be useful in the study of B- and T-cell malignancies.

Specimen Requirement

50 ng of high-quality genomic DNA.

Reference

1. EP Rock et al., J *Exp Med* 179 (1): 323-8 (1994).
2. JE Miller et al., *Molecular Genetic Pathology* (2nd ed.). Springer Science & Business Media. 2013: 30.2.7.13.

Simplified Representation of the *TRB* Gene

Depicted are the variable (Vβ), diversity (Dβ), and joining (Jβ) gene regions involved in *TRB* gene rearrangements, in addition to the downstream consensus (Cβ) gene regions.

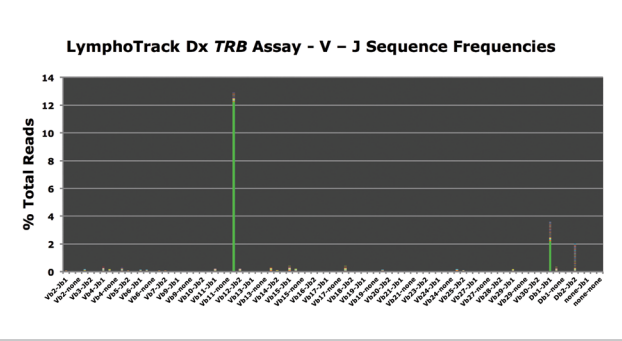
Reagents - MiSeq® Detection

Kit A Components

Master Mix Name	Index #
TRB MiSeq 01	A001
TRB MiSeq 02	A002
TRB MiSeq 03	A003
TRB MiSeq 04	A004
TRB MiSeq 05	A005
TRB MiSeq 06	A006
TRB MiSeq 07	A007
TRB MiSeq 08	A008
Controls	
TRB POS (+) Qty. 1	NGS NEG (-) Qty. 1

Panel Components (includes all master mixes from Kit A plus the items below)

Master Mix Name	Index #	Master Mix Name	Index #
TRB MiSeq 09	A009	*TRB* MiSeq 18	A018
TRB MiSeq 10	A010	*TRB* MiSeq 19	A019
TRB MiSeq 11	A011	*TRB* MiSeq 20	A020
TRB MiSeq 12	A012	*TRB* MiSeq 21	A021
TRB MiSeq 13	A013	*TRB* MiSeq 22	A022
TRB MiSeq 14	A014	*TRB* MiSeq 23	A023
TRB MiSeq 15	A015	*TRB* MiSeq 25	A025
TRB MiSeq 16	A016	*TRB* MiSeq 27	A027
Controls			
TRB POS (+) Qty. 3		NGS NEG (-) Qty. 3	



V-J Sequence Frequencies. The LymphoTrack Dx bioinformatics software provides a stacked bar graph depicting the relative frequencies for the 200 most prevalent rearrangements sequenced and identified in the sample. To learn more about the LymphoTrack Dx software, please refer to the LymphoTrack Dx Bioinformatics Software section.

Ordering Information

Catalog #	Products	Quantity Components
9-225-0009	LymphoTrack® Dx *TRB* Assay Kit A - MiSeq®	8 indices - 5 sequencing reactions each
9-225-0019	LymphoTrack® Dx *TRB* Assay Panel - MiSeq®	24 indices - 5 sequencing reactions each
9-500-0009	LymphoTrack® Dx Software - MiSeq®	1 CD complimentary with purchase

CE IVD These products are CE-IVD assays for *in vitro* diagnostic use.

LymphoTrack® Dx

LymphoTrack Dx Bioinformatics Software

Software Use

The LymphoTrack Dx Bioinformatics Software package is provided with each LymphoTrack Dx Assay to analyze raw FASTQ files for clonality analysis of single or multiple target data sets (*IGHV* Leader (Leader), *IGH* FR1, *IGH* FR2, *IGH* FR3, *IGK, TRB*, or *TRG*). For data generated with LymphoTrack Dx *IGHV* Leader or *IGH* FR1 Assays, the software provides additional information, including the rate of somatic hypermutation and whether a clone would be functional based upon the presence of a premature stop codon. The software can also predict whether an open reading frame would be in- or out-of-frame.

The provided software is composed of two distinct parts:

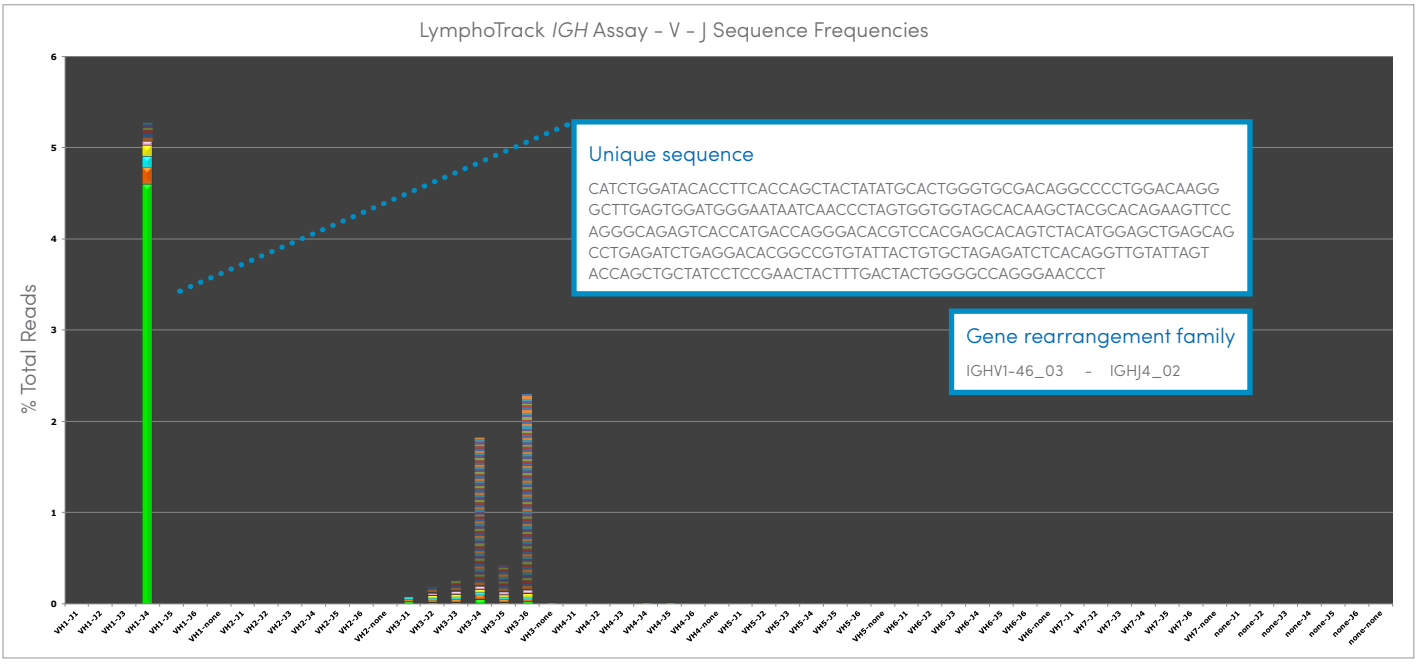
1. A bioinformatics Data Analysis Application
2. Microsoft Excel® Data Visualization *.xlsm* spreadsheets for each gene target

Minimum Software Requirements

- Processor: Intel Core 2 Duo or newer CPU recommended
- Hard Drive: At least 80 GB of free disk space is required; 250 GB recommended
- RAM: 4 GB required; 8 GB or more recommended
- Operating System: Windows 7 (64-bit) is required
- Java 7 for 64-bit operating systems or higher
- Microsoft Office Excel 2007, 2010, or 2013 with Macro support enabled is required for data visualization
- A CD-ROM drive*
- Microsoft Excel 2007/2010/2013 and Windows 7 configured for English (United States) language settings

* If a CD-ROM drive is not available, please contact us at: support@invivoscribe.com

Sequence Frequency Graph



The stacked bar graph depicts the top 200 sequencing reads for a sample. Each individual colored bar represents a unique population of cells. Different colors stacked at the same point on the x-axis represent unique sequences that utilize the same V and J gene families. The amplicons of these products vary in sequence and may also vary in product size.

Sequencing Summary

Using the merged read summary, along with the easy to follow flow charts in the accompanying LymphoTrack Dx Assay instructions for use (IFU), interpretation is quick and easy.

(1) Sample Name											
(1) Total reads = 32,458											
	(2)			(3)		(4)		(5)			
Rank	Sequence	Length	Merge count	V-gene	J-gene	% Total reads	Cumulative %	Mutation rate partial V-gene (%)	In-frame (Y/N)	No stop codon (Y/N)	V-coverage
1	TTCTCGTGGTG	455	29603	IGHV4-59_08	IGHJ4_02	9.93	9.93	11.26	Y	Y	98.63
2	CTCGCCCTCCT	463	205	IGHV5-51_01	IGHJ4_02	0.07	9.99	0.00	Y	Y	99.66
3	GGTTTTCCTTG	484	201	IGHV3-7_01	IGHJ4_02	0.07	10.06	7.77	Y	Y	100.00
4	CTCGCCCTCCT	463	185	IGHV5-51_01	IGHJ5_02	0.06	10.12	6.08	Y	Y	99.32
5	CTCGCCCTCCT	469	170	IGHV5-51_01	IGHJ4_02	0.06	10.18	0.00	Y	Y	99.32
6	CTCGCCCTCCT	466	160	IGHV5-51_01	IGHJ4_02	0.05	10.23	0.00	Y	Y	99.66
7	CTGCTGCTGAC	460	159	IGHV2-5_10	IGHJ5_02	0.05	10.29	8.08	Y	Y	97.64
8	GGTTTTCCTTG	493	156	IGHV3-48_02	IGHJ6_02	0.05	10.34	3.72	Y	Y	98.99
9	CTCGCCCTCCT	334	153	IGHV5-51_02	IGHJ2_01	0.05	10.39	3.72	Y	N	27.70
10	CTCGCCCTCCT	334	152	IGHV5-51_02	IGHJ2_01	0.05	10.44	3.38	Y	N	26.01

(1) The sample name is clearly identified and the total number of reads generated for the sample is provided. Following the IFU, it is easy to determine whether the data generated for a sample can be assessed for the presence or absence of clonality.

(2) The sequence of clonal populations is provided and populations are ranked from most abundant to least prevalent. Sequences that are within 1-2 basepairs of each other are automatically merged to account for possible sequencing errors and to improve the accuracy and ease of sample interpretation.

(3) Sequences are aligned with reference genes to allow for easy identification of specific types of gene rearrangements such as IGHV3-21, which is characteristic of some CLL cases.

(4) The percentage that a unique sequence contributes to the total number of reads for a sample is calculated. Following the guidelines in the IFU, samples can be interpreted for the evidence indicating the presence or absence of clonality.

(5) For the LymphoTrack Dx *IGHV* Leader and *IGH* FR1 Assays, the somatic hypermutation status of a sequence is automatically calculated by comparing the identified sequence with a germline reference. In addition, predictions on whether the sequence would be functional can be drawn by the provided information regarding the presence of a premature stop codon or an open reading frame that is out-of-frame.

Ordering Information

Catalog #	Products	Quantity Components
9-500-0009	LymphoTrack® Dx Software - MiSeq®	1 CD complimentary with kit purchase
9-500-0007	LymphoTrack® Dx Software - PGM™	1 CD complimentary with kit purchase

CE IVD These products are CE-IVD assays for *in vitro* diagnostic use.

Warranty and Liability

Invivoscribe Technologies® (Invivoscribe®) is committed to providing the highest quality products. Invivoscribe® warrants that the products meet or exceed the performance standards described in the Instructions For Use, as to products with such an insert. If a product is covered by product specifications and does not perform as specified, our policy is to replace the product or credit the full purchase price. No other warranties of any kind, expressed or implied, are provided by Invivoscribe®. Invivoscribe® liability shall not exceed the purchase price of the product. Invivoscribe® shall have no liability for direct, indirect, consequential or incidental damages arising from the use, results of use, or inability to use its products; product efficacy under purchaser controlled conditions in purchaser's laboratory must be established and continually monitored through purchaser defined and controlled processes including but not limited to testing of positive, negative, and blank controls every time a sample is tested. Ordering, acceptance and use of product constitutes purchaser acceptance of sole responsibility for assuring product efficacy and purchaser agreement to the limitation of liability set forth in this paragraph.

NOTICE: Many of the products listed in the section that follows may be covered by one or more of the following: United States Patent 7785783, and additional patents and patent applications pending in the United States and elsewhere. All of these patents and applications are licensed exclusively to Invivoscribe® worldwide (except Australia) across the field of hematopathology, including diagnostics, monitoring, prognosis and treatment. These products require nucleic acid amplification methods such as Polymerase Chain Reaction (PCR). No license under these patents to use amplification processes or enzymes is conveyed expressly or by implication to the purchaser by the purchase of these products.

Next-Generation Sequencing (NGS)

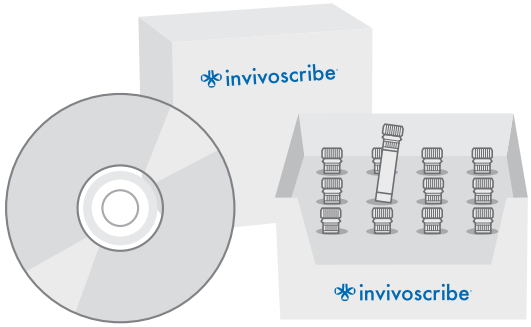
Research Use Only (RUO) Assays

LymphoTrack® Assays

LymphoTrack Assay kits are designed for the identification of gene rearrangements in hematologic samples utilizing next-generation sequencing (NGS) technologies. These assays take advantage of the wealth and depth of NGS data generated with either the Illumina® MiSeq® or the Thermo Fisher Scientific® Ion PGM™ platforms. They offer significant improvements over conventional clonality testing methods, by providing the distribution of gene rearrangements, DNA sequences, the mutational status (*IGH* only), and the ability to track specific gene rearrangements all with the same workflow.

LymphoTrack Assay kits offer a complete solution. All kits contain amplification master mixes and necessary controls. For a fast and easy workflow, primers include barcoded indices, which allow for a one-step PCR to generate sequence-ready amplicons and eliminate the need for a post-PCR ligation. By offering multiple kit configurations (8-24 indices for MiSeq®, 12 for Ion PGM™), Invivoscribe provides laboratories the ability to choose the appropriate kit for their sample throughput and read-depth requirements. Testing costs can be reduced by sequencing in a single run, with the possibility to combine: a) samples with different indices and b) amplicons from other LymphoTrack Assays. The included bioinformatics software sorts complex NGS data for easy analysis and visualization of individual samples.

Research Use Only (RUO) assays are not for sale in Europe and other global markets where equivalent CE-IVD assays are available and registered with the appropriate regulatory agencies. For more information regarding the CE-marked LymphoTrack® Dx Assays, please refer to the preceding pages.



Key Benefits

- One-step PCR for amplicon and library generation
- Identify and assess mutation status of B- and T-cell gene rearrangements
- Sequence amplicons from any LymphoTrack kit together
- Included bioinformatics software for easy analysis and interpretation
- Same reagents for clonality, somatic hypermutation (SHM), minimal residual disease (MRD) testing, and tracking/monitoring of immunotherapy constructs

LymphoTrack®
IGHV Leader Somatic Hypermutation Assay

LymphoTrack *IGHV* Leader Somatic Hypermutation Assay

Assay Uses

This research use only (RUO) assay for next-generation sequencing (NGS), identifies clonal *IGH* VH-JH rearrangements, the associated VH-JH DNA sequences, and the frequency distribution of VH region and JH region segment utilization. The assay also uses the Illumina® MiSeq® platform to define the extent of somatic hypermutation (SHM) present in the *IGHV* gene of analyzed samples.

*Note: If you would like to test for *IGHV* somatic hypermutation using the Thermo Fisher® Ion PGM™ platform please refer to the LymphoTrack *IGH* FR1 Assay (7-121-0007).

Summary and Explanation of the Test

The LymphoTrack *IGHV* Leader Somatic Hypermutation Assay for NGS provides significant improvements over clonality assays using fragment analysis and Sanger sequencing. The assay efficiently detects the majority of *IGH* gene rearrangements using a single multiplex master mix, identifies the DNA sequence specific for each clonal gene rearrangement, and calculates the degree of SHM for each sample.

The master mixes included in this assay target the Leader (VHL) and the joining (JH) gene regions of *IGH* and are designed with Illumina® adapters and indices (8 included in Kit A and 24 included in the Panel). Including adapters and indices in the primer design allows for a one-step PCR to generate sequence-ready amplicons, followed by direct pooling of samples for sequencing on a Illumina® MiSeq® flow cell.

The included LymphoTrack bioinformatics software enables simplified analysis and visualization of data generated from this assay.

Positive (clonal positive, SHM negative), negative (polyclonal), and SHM positive (clonal positive, SHM positive) controls are included in the kit. Primers included in the master mixes are designed with Illumina® adapters and indices (8 or 24 indices per framework kits) or Thermo Fisher adaptors and indices (12 indices per framework kits). This allows for a one-step PCR reaction to generate sequence-ready amplicons and pooling of several different samples on the same Illumina® MiSeq® flow cell or Ion PGM chip. The LymphoTrack bioinformatics software enables easy analysis and visualization of data and the LymphoTrack MRD Software allows sequences to be tracked in subsequent samples. Please see the LymphoTrack MRD software section to learn how the LymphoTrack Assays can be applied to MRD studies, or email MRD@invivoscribe.com.

Background

The human immunoglobulin heavy chain (*IGH*) gene locus on chromosome 14 (14q32.3) includes 46 - 52 functional and 30 nonfunctional variable (VH) gene segments, 27 functional diversity (DH) gene segments, and 6 functional joining (JH) gene segments spread over 1250 kilobases.

During B-cell development, genes encoding the IGH molecules are assembled from multiple polymorphic gene segments that undergo rearrangements and selection, generating VH-DH-JH combinations that are unique in both length and sequence for each cell.

Since leukemias and lymphomas originate from the malignant transformation of individual lymphoid cells, all leukemias and lymphomas generally share one or more cell-specific or "clonal" antigen receptor gene rearrangements. Therefore, tests that detect *IGH* clonal rearrangements can be useful in the study of B-cell malignancies. An additional level of diversity is generated in the antigen receptors by somatic point mutations in the variable regions and this mutation status provides important prognostic information for chronic lymphocytic leukemia (CLL) and small lymphocytic lymphoma (SLL). In addition, NGS methods can improve disease stratification and elucidate subclone gene profiles.

Specimen Requirement

50 ng of high-quality genomic DNA.

Reference

1. B Stamatopoulous et al., *Leukemia* 4:837-845 (2017).
2. F Davi et al., *Leukemia* 22: 212-214 (2008).
3. JE Miller et al., *Molecular Genetic Pathology* (2nd ed.). Springer Science & Business Media. 2013: 302.2.7.13 and 30.2.7.18.
4. KJ Trainor et al., *Blood* 75: 2220-2222 (1990).
5. P Ghia et al., *Leukemia* 21: 2-3 (2007).
6. P Ghia et al., *Blood* 105: 1678-685 (2005).
7. S Tonegawa. *Nature* 302: 575-581 (1983).

Simple representation of the organization of the immunoglobulin heavy chain (*IGH*) gene on chromosome 14. Depicted are the variable region (VH) genes and downstream consensus joining region genes (JH) that are involved in rearrangements. Upstream of the variable gene segments, the leader sequence (VHL) is also depicted. Diversity region genes are not depicted.

Reagents - MiSeq® Detection

Kit A Components

Master Mix Name	Index #
IGH Leader MiSeq 01	A001
IGH Leader MiSeq 02	A002
IGH Leader MiSeq 03	A003
IGH Leader MiSeq 04	A004
IGH Leader MiSeq 05	A005
IGH Leader MiSeq 06	A006
IGH Leader MiSeq 07	A007
IGH Leader MiSeq 08	A008
Controls	
IGH SHM POS (+) Qty. 1	
IGH POS (+) Qty. 1	
NGS NEG (-) Qty. 1	

Panel Components (includes all master mixes from Kit A plus the items below)

Master Mix Name	Index #	Master Mix Name	Index #
IGH Leader MiSeq 09	A009	*IGH* Leader MiSeq 18	A018
IGH Leader MiSeq 10	A010	*IGH* Leader MiSeq 19	A019
IGH Leader MiSeq 11	A011	*IGH* Leader MiSeq 20	A020
IGH Leader MiSeq 12	A012	*IGH* Leader MiSeq 21	A021
IGH Leader MiSeq 13	A013	*IGH* Leader MiSeq 22	A022
IGH Leader MiSeq 14	A014	*IGH* Leader MiSeq 23	A023
IGH Leader MiSeq 15	A015	*IGH* Leader MiSeq 25	A025
IGH Leader MiSeq 16	A016	*IGH* Leader MiSeq 27	A027
Controls			
IGH SHM POS (+) Qty. 3			
IGH POS (+) Qty. 3			
NGS NEG (-) Qty. 3			

Rank	Sequence	Length	Merge count	V-gene	J-gene	% Total reads	Cumulative %	Mutation rate partial V-gene (%)	In-frame (Y/N)	No stop codon (Y/N)	V-coverage
1	TTCTCGTGGTG	455	29603	IGHV4-59_08	IGHJ4_02	9.93	9.93	11.26	Y	Y	98.63
2	CTCGCCCTCCT	463	205	IGHV5-51_01	IGHJ4_02	0.07	9.99	0.00	Y	Y	99.66
3	GGTTTTCCTTG	484	201	IGHV3-7_01	IGHJ4_02	0.07	10.06	7.77	Y	Y	100.00
4	CTCGCCCTCCT	463	185	IGHV5-51_01	IGHJ5_02	0.06	10.12	6.08	Y	Y	99.32
5	CTCGCCCTCCT	469	170	IGHV5-51_01	IGHJ4_02	0.06	10.18	0.00	Y	Y	99.32
6	CTCGCCCTCCT	466	160	IGHV5-51_01	IGHJ4_02	0.05	10.23	0.00	Y	Y	99.66
7	CTGCTGCTGAC	460	159	IGHV2-5_10	IGHJ5_02	0.05	10.29	8.08	Y	Y	97.64
8	GGTTTTCCTTG	493	156	IGHV3-48_02	IGHJ6_02	0.05	10.34	3.72	Y	Y	98.99
9	CTCGCCCTCCT	334	153	IGHV5-51_02	IGHJ2_01	0.05	10.39	3.72	Y	N	27.70
10	CTCGCCCTCCT	334	152	IGHV5-51_02	IGHJ2_01	0.05	10.44	3.38	Y	N	26.01

Example Data. Depicted are the top 10 sequences from a read summary generated by the LymphoTrack Software - MiSeq®. Highlighted columns represent fields that are unique to SHM analysis and include the SHM mutation rate and predictions pertaining to whether a sequence is in-frame or contains a premature stop codon. To learn more about the LymphoTrack software, please refer to the LymphoTrack Bioinformatics Software section.

Ordering Information

Catalog #	Products	Quantity Components
7-121-0059	LymphoTrack® *IGHV* Leader Somatic Hypermutation Assay Kit A - MiSeq®	8 indices - 5 sequencing reactions each
7-121-0069	LymphoTrack® *IGHV* Leader Somatic Hypermutation Assay Panel - MiSeq®	24 indices - 5 sequencing reactions each
7-500-0009	LymphoTrack® Software - MiSeq®	1 CD complimentary with purchase

LymphoTrack®
IGH Assays

LymphoTrack *IGH* FR1/FR2/FR3 Assays

Assay Uses

These research use only (RUO) assays for next-generation sequencing (NGS), identify clonal *IGH* VH-JH rearrangements, the associated VH-JH region DNA sequences, the frequency distribution of VH region and JH region segment utilization, as well as the degree of somatic hypermutation (SHM) of rearranged genes using the Illumina® MiSeq® or Thermo Fisher Scientific® Ion PGM™. The LymphoTrack *IGH* FR1, *IGH* FR2, *IGH* FR3 Assays contain primers that target the conserved framework 1 (FR1), framework 2 (FR2), and framework 3 (FR3) regions, respectively. The LymphoTrack *IGH* FR1/2/3 Assay kits contain the master mixes of all three frameworks.

Summary and Explanation of the Test

The LymphoTrack *IGH* Assays represent a significant improvement over clonality assays that utilize fragment analysis by providing four important and complementary uses:

1. Detection of clonal populations.
2. Identification of sequence information and VH-JH segment utilization.
3. The LymphoTrack *IGH* FR1 Assays provide the degree of SHM of the immunoglobulin variable heavy chain (*IGHV*).
4. The ability to track sequences in subsequent samples with the Invivoscribe LymphoTrack MRD Software.

Primers included in the master mixes are designed with Illumina® adapters and indices (8 or 24 indices per framework kits) or Thermo Fisher adaptors and indices (12 indices per framework kits). This allows for a one-step PCR reaction to generate sequence-ready amplicons and pooling of several different samples on the same Illumina® MiSeq® flow cell or Ion PGM chip. The LymphoTrack bioinformatics software enables easy analysis and visualization of data and the LymphoTrack MRD Software allows sequences to be tracked in subsequent samples. Please see the LymphoTrack MRD software section to learn how the LymphoTrack Assays can be applied to MRD studies, or email MRD@invivoscribe.com.

Positive clonal (SHM negative) and negative polyclonal DNA controls are included in kits. A clonal SHM positive control can be purchased separately (4-088-0008).

Background

The human immunoglobulin heavy chain (*IGH*) gene locus on chromosome 14 (14q32.3) includes 46-52 functional and 30 nonfunctional variable (VH) gene segments, 27 functional diversity (DH) gene segments, and 6 functional joining (JH) gene segments spread over 1250 kilobases.

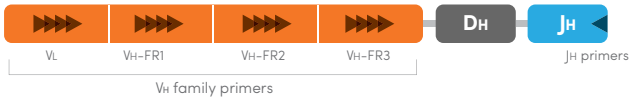
During development of lymphoid cells, the antigen receptor genes go through somatic gene rearrangements. For example, during B-cell development, genes encoding the IGH molecules are assembled from multiple polymorphic gene segments that undergo rearrangements and selection, generating VH-DH-JH combinations. Since leukemias and lymphomas originate from the malignant transformation of individual lymphoid cells, all leukemias and lymphomas generally share one or more cell-specific or "clonal" antigen receptor gene rearrangements. Therefore, tests that detect *IGH* clonal populations can be useful in the study of B- and T-cell malignancies.

Specimen Requirement

50 ng of high-quality genomic DNA.

Reference

1. S Tonegawa. *Nature* 302: 575-581 (1983).
2. JE Miller et al., *Molecular Genetic Pathology* (2nd ed.). Springer Science & Business Media. 2013: 30.2.7.13 and 30.2.7.18.
3. KJ Trainor et al., *Blood* 75: 2220-2222 (1990).
4. P Ghia. *Leukemia* 21: 1-3 (2007).



Simple representation of the organization of the immunoglobulin heavy chain (*IGH*) gene locus on chromosome 14. Depicted are the variable region (VH) genes and downstream consensus joining region genes segments (JH) that are involved in rearrangements.

Reagents - MiSeq® Detection

The LymphoTrack *IGH* FR1/2/3 Assays contain components from respective individual FR Kit A's or Panels.

LymphoTrack *IGH* FR1 Components			LymphoTrack *IGH* FR2 Components			LymphoTrack *IGH* FR3 Components		
Master Mix Name	Index #		Master Mix Name	Index #		Master Mix Name	Index #	
IGH FR1 MiSeq 01	A001	KIT A	*IGH* FR2 MiSeq 01	A001	KIT A	*IGH* FR3 MiSeq 01	A001	KIT A
IGH FR1 MiSeq 02	A002		*IGH* FR2 MiSeq 02	A002		*IGH* FR3 MiSeq 02	A002	
IGH FR1 MiSeq 03	A003		*IGH* FR2 MiSeq 03	A003		*IGH* FR3 MiSeq 03	A003	
IGH FR1 MiSeq 04	A004		*IGH* FR2 MiSeq 04	A004		*IGH* FR3 MiSeq 04	A004	
IGH FR1 MiSeq 05	A005		*IGH* FR2 MiSeq 05	A005		*IGH* FR3 MiSeq 05	A005	
IGH FR1 MiSeq 06	A006		*IGH* FR2 MiSeq 06	A006		*IGH* FR3 MiSeq 06	A006	
IGH FR1 MiSeq 07	A007		*IGH* FR2 MiSeq 07	A007		*IGH* FR3 MiSeq 07	A007	
IGH FR1 MiSeq 08	A008		*IGH* FR2 MiSeq 08	A008		*IGH* FR3 MiSeq 08	A008	
IGH FR1 MiSeq 09	A009		*IGH* FR2 MiSeq 09	A009		*IGH* FR3 MiSeq 09	A009	
IGH FR1 MiSeq 10	A010		*IGH* FR2 MiSeq 10	A010		*IGH* FR3 MiSeq 10	A010	
IGH FR1 MiSeq 11	A011		*IGH* FR2 MiSeq 11	A011		*IGH* FR3 MiSeq 11	A011	
IGH FR1 MiSeq 12	A012		*IGH* FR2 MiSeq 12	A012		*IGH* FR3 MiSeq 12	A012	
IGH FR1 MiSeq 13	A013		*IGH* FR2 MiSeq 13	A013		*IGH* FR3 MiSeq 13	A013	
IGH FR1 MiSeq 14	A014		*IGH* FR2 MiSeq 14	A014		*IGH* FR3 MiSeq 14	A014	
IGH FR1 MiSeq 15	A015		*IGH* FR2 MiSeq 15	A015		*IGH* FR3 MiSeq 15	A015	
IGH FR1 MiSeq 16	A016		*IGH* FR2 MiSeq 16	A016		*IGH* FR3 MiSeq 16	A016	
IGH FR1 MiSeq 18	A018		*IGH* FR2 MiSeq 18	A018		*IGH* FR3 MiSeq 18	A018	
IGH FR1 MiSeq 19	A019		*IGH* FR2 MiSeq 19	A019		*IGH* FR3 MiSeq 19	A019	
IGH FR1 MiSeq 20	A020		*IGH* FR2 MiSeq 20	A020		*IGH* FR3 MiSeq 20	A020	
IGH FR1 MiSeq 21	A021		*IGH* FR2 MiSeq 21	A021		*IGH* FR3 MiSeq 21	A021	
IGH FR1 MiSeq 22	A022		*IGH* FR2 MiSeq 22	A022		*IGH* FR3 MiSeq 22	A022	
IGH FR1 MiSeq 23	A023		*IGH* FR2 MiSeq 23	A023		*IGH* FR3 MiSeq 23	A023	
IGH FR1 MiSeq 25	A025		*IGH* FR2 MiSeq 25	A025		*IGH* FR3 MiSeq 25	A025	
IGH FR1 MiSeq 27	A027		*IGH* FR2 MiSeq 27	A027		*IGH* FR3 MiSeq 27	A027	

Kit A's contain indices *IGH* FRX A001 to A008. Panels contain all master mixes listed above.

Controls in Individual FR (1, 2, or 3) Kit A's	Controls in Individual FR (1,2, or 3) Panels	Controls in Combo FR 1/2/3 Kit A	Controls in Combo FR 1/2/3 Panel
IGH POS (+) Qty. 1	*IGH* POS (+) Qty. 3	*IGH* POS (+) Qty. 2	*IGH* POS (+) Qty. 6
NGS NEG (-) Qty. 1	NGS NEG (-) Qty. 3	NGS NEG (-) Qty. 2	NGS NEG (-) Qty. 6

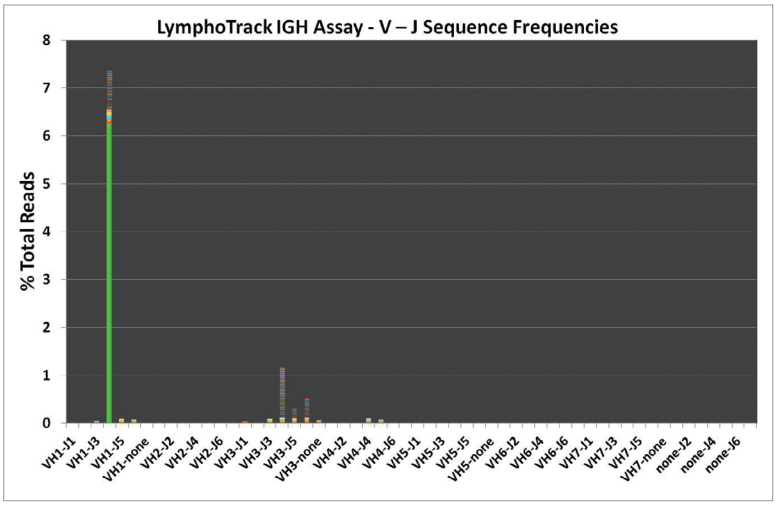
LymphoTrack *IGH* FR1/FR2/FR3 Assays cont.

Reagents - Ion PGM™ Detection

The LymphoTrack *IGH* FR1/2/3 Assays contain components from respective individual FR Assays.

LymphoTrack *IGH* FR1 Components		LymphoTrack *IGH* FR2 Components		LymphoTrack *IGH* FR3 Components	
Master Mix Name	Index #	Master Mix Name	Index #	Master Mix Name	Index #
IGH FR1 PGM 01	IonXpress_001	*IGH* FR2 PGM 01	IonXpress_001	*IGH* FR3 PGM 01	IonXpress_001
IGH FR1 PGM 02	IonXpress_002	*IGH* FR2 PGM 02	IonXpress_002	*IGH* FR3 PGM 02	IonXpress_002
IGH FR1 PGM 03	IonXpress_003	*IGH* FR2 PGM 03	IonXpress_003	*IGH* FR3 PGM 03	IonXpress_003
IGH FR1 PGM 04	IonXpress_004	*IGH* FR2 PGM 04	IonXpress_004	*IGH* FR3 PGM 04	IonXpress_004
IGH FR1 PGM 07	IonXpress_007	*IGH* FR2 PGM 07	IonXpress_007	*IGH* FR3 PGM 07	IonXpress_007
IGH FR1 PGM 08	IonXpress_008	*IGH* FR2 PGM 08	IonXpress_008	*IGH* FR3 PGM 08	IonXpress_008
IGH FR1 PGM 09	IonXpress_009	*IGH* FR2 PGM 09	IonXpress_009	*IGH* FR3 PGM 09	IonXpress_009
IGH FR1 PGM 10	IonXpress_010	*IGH* FR2 PGM 10	IonXpress_010	*IGH* FR3 PGM 10	IonXpress_010
IGH FR1 PGM 11	IonXpress_011	*IGH* FR2 PGM 11	IonXpress_011	*IGH* FR3 PGM 11	IonXpress_011
IGH FR1 PGM 12	IonXpress_012	*IGH* FR2 PGM 12	IonXpress_012	*IGH* FR3 PGM 12	IonXpress_012
IGH FR1 PGM 13	IonXpress_013	*IGH* FR2 PGM 13	IonXpress_013	*IGH* FR3 PGM 13	IonXpress_013
IGH FR1 PGM 14	IonXpress_014	*IGH* FR2 PGM 14	IonXpress_014	*IGH* FR3 PGM 14	IonXpress_014

Controls in Individual FR (1,2, or 3) Kits	Controls in FR 1/2/3 Kit
IGH POS (+) Qty. 2	*IGH* POS (+) Qty. 4
NGS NEG (-) Qty. 2	NGS NEG (-) Qty. 4



V-J Sequence Frequencies. The LymphoTrack Software provides a stacked bar graph depicting the relative frequencies for the 200 most prevalent VH–JH rearrangements identified in a sample. To learn more about the LymphoTrack software, please refer to the LymphoTrack Bioinformatics Software section.

Ordering Information

Catalog #	Products	Quantity Components
7-121-0129	LymphoTrack® *IGH* FR1/2/3 Assay Kit A - MiSeq®	8 + 8 + 8 indices - 5 sequencing reactions each
7-121-0139	LymphoTrack® *IGH* FR1/2/3 Assay Panel - MiSeq®	24 + 24 + 24 indices - 5 sequencing reactions each
7-121-0009	LymphoTrack® *IGH* FR1 Assay Kit A - MiSeq®	8 indices - 5 sequencing reactions each
7-121-0039	LymphoTrack® *IGH* FR1 Assay Panel - MiSeq®	24 indices - 5 sequencing reactions each
7-121-0089	LymphoTrack® *IGH* FR2 Assay Kit A - MiSeq®	8 indices - 5 sequencing reactions each
7-121-0099	LymphoTrack® *IGH* FR2 Assay Panel - MiSeq®	24 indices - 5 sequencing reactions each
7-121-0109	LymphoTrack® *IGH* FR3 Assay Kit A - MiSeq®	8 indices - 5 sequencing reactions each
7-121-0119	LymphoTrack® *IGH* FR3 Assay Panel - MiSeq®	24 indices - 5 sequencing reactions each
7-500-0009	LymphoTrack® Software - MiSeq®	1 CD complimentary with purchase
7-121-0057	LymphoTrack® *IGH* FR1/2/3 Assay - PGM™	12 + 12 + 12 indices - 5 sequencing reactions each
7-121-0007	LymphoTrack® *IGH* FR1 Assay - PGM™	12 indices - 5 sequencing reactions each
7-121-0037	LymphoTrack® *IGH* FR2 Assay - PGM™	12 indices - 5 sequencing reactions each
7-121-0047	LymphoTrack® *IGH* FR3 Assay - PGM™	12 indices - 5 sequencing reactions each
7-500-0007	LymphoTrack® Software - PGM™	1 CD complimentary with purchase
7-500-0008	LymphoTrack® MRD Software* **NEW**	1 CD complimentary with purchase

* MRD Software can be used to track sequences generated by either LymphoTrack Assays - MiSeq® or Ion PGM™.

LymphoTrack®
IGK Assay

LymphoTrack *IGK* Assays

Assay Uses

This research use only (RUO) assay for next-generation sequencing (NGS), identifies clonal *IGK* Vκ-Jκ, Vκ-Kde, and intron-Kde (INTR-Kde) rearrangements, the corresponding DNA sequences, and provides the distribution frequency of Vκ-Jκ, Vκ-Kde, and INTR-Kde segment utilization using the Illumina® MiSeq® or Thermo Fisher Scientific® Ion PGM™ platforms.

Summary and Explanation of the Test

The LymphoTrack *IGK* Assay represents a significant improvement over clonality assays that utilize fragment analysis by providing three important and complementary uses:

1. Detection of clonal populations.
2. Identification of sequence information and gene segment utilization.
3. Ability to track sequences in subsequent samples with the use of the LymphoTrack MRD Software.

Unlike conventional fragment analysis assays, this NGS method utilizes a single multiplex master mix to target conserved regions of *IGK* that are described in lymphoid malignancies. Primers are designed with Illumina® adapters and indices (8-24) or Thermo Fisher Scientific adaptors and indices (12), thereby allowing for a one-step PCR reaction to generate sequence-ready amplicons. To reduce per sample testing costs, amplicons from different samples (amplified with different indexed master mixes) or LymphoTrack kits can be sequenced together on a single Illumina® MiSeq® flow cell or Ion PGM chip.

The LymphoTrack bioinformatics software enables simplified analysis and visualization of data and the LymphoTrack MRD Software allows identified sequences to be tracked in subsequent samples. Please see the LymphoTrack MRD software section to learn how the LymphoTrack Assays can be applied to MRD studies, or email MRD@invivoscribe.com. Positive (clonal) and negative (polyclonal) DNA controls are included in the kits.

Background

The human immunoglobulin kappa (*IGK*) gene locus on chromosome 2 (2p11.2) includes 76 V (variable) genes spanning 7 subgroups and 5 J (joining) gene segments upstream of the Cκ region. The Kde, approximately 24 kb downstream of the Jκ-Cκ region, can also rearrange with Vκ gene segments and the isolated recombination signal sequence in the Jκ-Cκ intronic region.

During development of lymphoid cells, antigen receptor genes undergo somatic gene rearrangements. Specifically during B-cell development, genes encoding IGK molecules are assembled from multiple polymorphic gene segments that undergo rearrangements generating V-J combinations unique in both length and sequence. Since leukemias and lymphomas originate from the malignant transformation of individual lymphoid cells, all leukemias and lymphomas generally share one or more cell-specific or "clonal" antigen receptor gene rearrangements. Therefore, tests that detect *IGK* clonal populations can be useful in the study of B- and T-cell malignancies and complement *IGH* testing, as the *IGK* receptor is less susceptible to somatic mutations.

Specimen Requirement

50 ng of high-quality genomic DNA.

Reference

1. S Tonegawa et al., *Nature* 302: 575-581 (1983).
2. JE Miller et al., *Molecular Genetic Pathology* (2nd ed.). Springer Science & Business Media. 2013: 30.2.7.13 and 30.2.7.18.
3. KJ Trainor et al., *Blood* 75: 2220-2222 (1990).

Simplified Representation of the *IGK* Gene

Depicted are the variable region (Vκ) genes or variable intragenic Jκ-Cκ intron (Jκ-Cκ INTR) and downstream consensus joining region genes (Jκ) or kappa deleting element (Kde) that are involved in *IGK* gene rearrangements.

Reagents - MiSeq® Detection

Kit A Components

Master Mix Name	Index #
IGK MiSeq 01	A001
IGK MiSeq 02	A002
IGK MiSeq 03	A003
IGK MiSeq 04	A004
IGK MiSeq 05	A005
IGK MiSeq 06	A006
IGK MiSeq 07	A007
IGK MiSeq 08	A008

Controls	
IGK POS (+) Qty. 1	NGS NEG (-) Qty. 1

Panel Components (includes all master mixes from Kit A plus the items below)

Master Mix Name	Index #	Master Mix Name	Index #
IGK MiSeq 09	A009	*IGK* MiSeq 18	A018
IGK MiSeq 10	A010	*IGK* MiSeq 19	A019
IGK MiSeq 11	A011	*IGK* MiSeq 20	A020
IGK MiSeq 12	A012	*IGK* MiSeq 21	A021
IGK MiSeq 13	A013	*IGK* MiSeq 22	A022
IGK MiSeq 14	A014	*IGK* MiSeq 23	A023
IGK MiSeq 15	A015	*IGK* MiSeq 25	A025
IGK MiSeq 16	A016	*IGK* MiSeq 27	A027

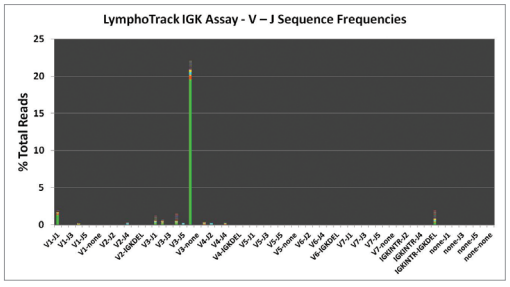
Controls	
IGK POS (+) Qty. 3	NGS NEG (-) Qty. 3

Reagents - Ion PGM™ Detection

Assay Components

Master Mix Name	Index #	Master Mix Name	Index #
IGK PGM 01	IonXpress_001	*IGK* PGM 11	IonXpress_011
IGK PGM 02	IonXpress_002	*IGK* PGM 12	IonXpress_012
IGK PGM 04	IonXpress_004	*IGK* PGM 13	IonXpress_013
IGK PGM 08	IonXpress_008	*IGK* PGM 14	IonXpress_014
IGK PGM 09	IonXpress_009	*IGK* PGM 16	IonXpress_016
IGK PGM 010	IonXpress_010	*IGK* PGM 17	IonXpress_017

Controls	
IGK POS (+) Qty. 2	NGS NEG (-) Qty. 2



V-J Sequence Frequencies. The LymphoTrack bioinformatics software provides a stacked bar graph depicting the relative frequencies for the 200 most prevalent rearrangements sequenced and identified in the sample. To learn more about the LymphoTrack software, please refer to the LymphoTrack Bioinformatics Software section.

Ordering Information

Catalog #	Products	Quantity Components
7-122-0009	LymphoTrack® *IGK* Assay Kit A - MiSeq®	8 indices - 5 sequencing reactions each
7-122-0019	LymphoTrack® *IGK* Assay Panel - MiSeq®	24 indices - 5 sequencing reactions each
7-500-0009	LymphoTrack® Software - MiSeq®	1 CD complimentary with purchase
7-122-0007	LymphoTrack® *IGK* Assay - PGM™	12 indices - 5 sequencing reactions each
7-500-0007	LymphoTrack® Software - PGM™	1 CD complimentary with purchase
7-500-0008	LymphoTrack® MRD Software* NEW	1 CD complimentary with purchase

* MRD Software can be used to track sequences generated by either LymphoTrack Assays - MiSeq® or Ion PGM™.

LymphoTrack®
TRG Assay

LymphoTrack *TRG* Assays

Assay Uses

This research use only (RUO) assay for next-generation sequencing (NGS) identifies clonal *TRG* V-J rearrangements and the associated V-J region DNA sequences. It also provides the frequency distribution of V-J segment utilization using the Illumina® MiSeq® or Thermo Fisher Scientific® Ion PGM™.

Summary and Explanation of the Test

The LymphoTrack *TRG* Assay represents a significant improvement over existing clonality assays that utilize fragment analysis by providing three important and complementary uses:

1. Detection of clonal populations.
2. Identification of sequence information and gene segment utilization.
3. Ability to track sequences in subsequent samples with the use of the Invivoscribe MRD Software.

The single multiplex master mix targets all conserved regions within the variable (V) and the joining (J) genes described in lymphoid malignancies. The average PCR amplicon size of the *TRG* gene rearrangements generated using this assay was designed to be compatible with fragmented DNA isolated from challenging samples such as FFPE sections.

Primers are designed with Illumina® adapters and indices (8-24) or Thermo Fisher Scientific adaptors and indices (12), thereby allowing for a one-step PCR reaction to generate sequence-ready amplicons. In addition, amplicons from different samples (amplified with different indexed master mixes) or LymphoTrack kits can be sequenced together on a single Illumina® MiSeq® flow cell or Ion PGM chip to reduce per sample testing costs.

The LymphoTrack bioinformatics software enables simplified analysis and visualization of data generated from this assay and the LymphoTrack MRD Software allows sequences to be tracked in subsequent samples. Please see the LymphoTrack MRD software section to learn how the LymphoTrack Assays can be applied to MRD studies, or email MRD@invivoscribe.com. Positive (clonal) and negative (polyclonal) DNA controls are included in the kits.

Background

The human T-cell receptor gamma (*TRG*) gene locus on chromosome 7 (7q14) includes 14 variable region (Group I, II, III, and IV), 5 joining region (J) gene segments, and 2 constant (C) genes spread over 200 kilobases.

During development of lymphoid cells, the antigen receptor genes (including gene segments within the *TRG* locus), undergo somatic gene rearrangement. These developmentally regulated gene rearrangements generate V-J combinations that are unique for each cell. Since leukemias and lymphomas originate from the malignant transformation of individual lymphoid cells, all leukemias and lymphomas generally share one or more cell-specific or "clonal" antigen receptor gene rearrangements. Therefore, tests that detect *TRG* clonal populations can be useful in the study of T-cell and certain B-cell malignancies. Since the *TRG* locus rearranges before the *TRB* locus, and all unsuccessful or unproductive rearrangements are retained in the cells, examination of the *TRG* locus can identify both clonal d/g as well as clonal a/b T-cells. Clonal a/b T-cells would be expected to have biallelic *TRG* gene rearrangements.

Specimen Requirement

50 ng of high-quality genomic DNA.

Reference

1. S Tonegawa et al., *Nature* 302: 575-581 (1983).
2. JE Miller et al., *Molecular Genetic Pathology* (2nd ed.). Springer Science & Business Media. 2013: 30.2.7.13 and 30.2.7.18.
3. KJ Trainor et al., *Blood* 75: 2220-2222 (1990).

Simplified Representation of the *TRG* Gene

Simple representation of the organization of the T-cell receptor gamma gene on chromosome 7. Depicted are the variable region genes (Vγ2–Vγ11) and downstream joining region genes (Jγ1/2–JγP1/2) that are involved in rearrangements in T-cell lymphomas.

Reagents - MiSeq® Detection

Kit A Components

Master Mix Name	Index #
TRG MiSeq 01	A001
TRG MiSeq 02	A002
TRG MiSeq 03	A003
TRG MiSeq 04	A004
TRG MiSeq 05	A005
TRG MiSeq 06	A006
TRG MiSeq 07	A007
TRG MiSeq 08	A008

Controls	
TRG POS (+) Qty. 1	NGS NEG (-) Qty. 1

Panel Components (includes all master mixes from Kit A plus the items below)

Master Mix Name	Index #	Master Mix Name	Index #
TRG MiSeq 09	A009	*TRG* MiSeq 18	A018
TRG MiSeq 10	A010	*TRG* MiSeq 19	A019
TRG MiSeq 11	A011	*TRG* MiSeq 20	A020
TRG MiSeq 12	A012	*TRG* MiSeq 21	A021
TRG MiSeq 13	A013	*TRG* MiSeq 22	A022
TRG MiSeq 14	A014	*TRG* MiSeq 23	A023
TRG MiSeq 15	A015	*TRG* MiSeq 25	A025
TRG MiSeq 16	A016	*TRG* MiSeq 27	A027

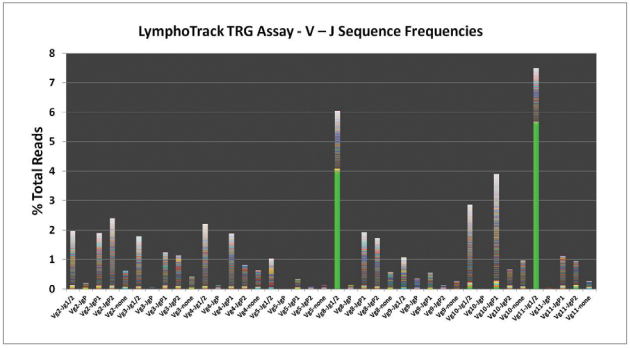
Controls	
TRG POS (+) Qty. 3	NGS NEG (-) Qty. 3

Reagents - Ion PGM™ Detection

Assay Components

Master Mix Name	Index #	Master Mix Name	Index #	Master Mix Name	Index #
TRG PGM 01	IonXpress_001	*TRG* PGM 07	IonXpress_007	*TRG* PGM 11	IonXpress_011
TRG PGM 02	IonXpress_002	*TRG* PGM 08	IonXpress_008	*TRG* PGM 12	IonXpress_012
TRG PGM 03	IonXpress_003	*TRG* PGM 09	IonXpress_009	*TRG* PGM 13	IonXpress_013
TRG PGM 04	IonXpress_004	*TRG* PGM 10	IonXpress_010	*TRG* PGM 14	IonXpress_014

Controls	
TRG POS (+) Qty. 2	NGS NEG (-) Qty. 2



V - J Sequence Frequencies. The LymphoTrack bioinformatics software provides a stacked bar graph depicting the relative frequencies for the V-J rearrangements identified and sequenced in a sample. To learn more about the LymphoTrack software, please refer to the LymphoTrack Bioinformatics Software section.

Ordering Information

Catalog #	Products	Quantity Components
7-227-0019	LymphoTrack® *TRG* Assay Kit A - MiSeq®	8 indices - 5 sequencing reactions each
7-227-0009	LymphoTrack® *TRG* Assay Panel - MiSeq®	24 indices - 5 sequencing reactions each
7-500-0009	LymphoTrack® Software - MiSeq®	1 CD complimentary with purchase
7-227-0007	LymphoTrack® *TRG* Assay - PGM™	12 indices - 5 sequencing reactions each
7-500-0007	LymphoTrack® Software - PGM™	1 CD complimentary with purchase
7-500-0008	LymphoTrack® MRD Software* NEW	1 CD complimentary with purchase

* MRD Software can be used to track sequences generated by either LymphoTrack Assays - MiSeq® or Ion PGM™.

LymphoTrack®
TRB Assay

LymphoTrack *TRB* Assays

Assay Uses

This research use only (RUO) assay for next-generation sequencing (NGS) identifies clonal *TRB* Vβ-(Dβ-)Jβ rearrangements, the associated Vβ-(Dβ-)Jβ region DNA sequences, it provides the frequency distribution of Vβ, Dβ, and Jβ region segment utilization using the Illumina® MiSeq® platform.

Analysis of the *TRB* locus increases the probability of identifying T cell receptor gene rearrangements, as compared to testing for *TRG* gene rearrangements only. As a result, combining the analysis of *TRB* and *TRG* loci increases the sensitivity of clonality detection.

Summary and Explanation of the Test

In contrast to the conventional fragment analysis assays for clonality that utilize three master mixes, this NGS-based assay contains a single multiplex master mix to target conserved V, D, and J regions of the *TRB* gene locus described in lymphoid malignancies. This reduces DNA sample requirements and simplifies the testing workflow. The LymphoTrack *TRB* master mix primers are also designed with Illumina® adapters and 8 indices (Kit A) or 24 indices (Panel). This allows amplicons generated from different indexed *TRB* master mixes to be pooled into a single sequencing library.

The associated LymphoTrack Software is capable of sorting complex NGS data by gene target. This offers a second layer of multiplexing to reduce per sample testing costs by allowing amplicons from any LymphoTrack Assay (*e.g. IGH, IGK, TRB, TRG*) to be sequenced on the same flow cell. In addition, the LymphoTrack Software provides easy visualization of data and the LymphoTrack Minimal Residual Disease Data Analysis Tool allows identified sequences to be tracked and monitored in subsequent samples. Positive clonal and negative polyclonal DNA controls are included in kits. Please see the LymphoTrack MRD software section to learn how the LymphoTrack Assays can be applied to MRD studies, or email MRD@invivoscribe.com.

Background

The LymphoTrack *TRB* Assay represent a significant improvement over clonality assessment by fragment analysis by providing two important and complementary uses:

1. Detection of initial clonal populations.
2. Identification of sequence information required to track clonal rearrangements in subsequent samples.

The human T cell receptor beta (*TRB*) gene locus on chromosome 7 (7q34) includes 65 Vβ (variable) gene segments, followed by two separate clusters of genes each containing a Dβ (diversity) gene, several Jβ (joining) genes, and a Cβ (constant) region spread over 685 kilobases. The 2 Cβ genes, *TRBC1* and *TRBC2*, encode highly homologous products with no functional difference.

During lymphoid cell development, antigen receptor genes undergo somatic gene rearrangements. Specifically, during T-cell development, genes encoding TRB molecules are assembled from multiple polymorphic gene segments that generate Vβ-Dβ-Jβ combinations unique in both length and sequence.

Since leukemias and lymphomas originate from the malignant transformation of individual lymphoid cells, all leukemias and lymphomas generally share one or more cell-specific or "clonal" antigen receptor gene rearrangements. Therefore, tests that detect *TRB* clonal rearrangements can be useful in the study of B- and T-cell malignancies.

Specimen Requirement

50 ng of high-quality genomic DNA.

Reference

3. EP Rock et al., J Exp Med 179 (1): 323-8 (1994).
4. JE Miller et al., Molecular Genetic Pathology (2nd Ed., sections 30.2.7.13 and 30.2.7.18). New York, USA: Springer Science & Business Media (2013).

Simplified Representation of the *TRB* Gene

Depicted are the variable (Vβ), diversity (Dβ), and joining (Jβ) gene regions involved in *TRB* gene rearrangements, in addition to the downstream consensus (Cβ) gene regions.

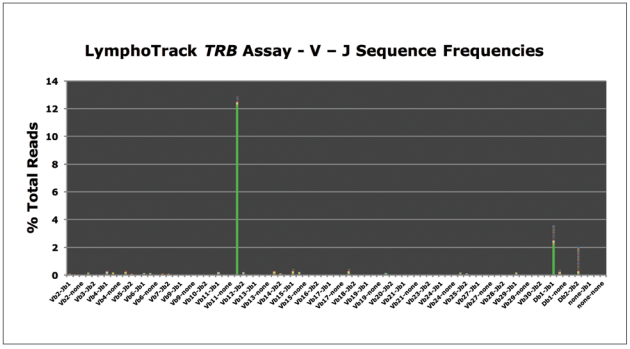
Reagents - MiSeq® Detection

Kit A Components

Master Mix Name	Index #
TRB MiSeq 01	A001
TRB MiSeq 02	A002
TRB MiSeq 03	A003
TRB MiSeq 04	A004
TRB MiSeq 05	A005
TRB MiSeq 06	A006
TRB MiSeq 07	A007
TRB MiSeq 08	A008
Controls	
TRB POS (+) Qty. 1	NGS NEG (-) Qty. 1

Panel Components (includes all master mixes from Kit A plus the items below)

Master Mix Name	Index #	Master Mix Name	Index #
TRB MiSeq 09	A009	*TRB* MiSeq 18	A018
TRB MiSeq 10	A010	*TRB* MiSeq 19	A019
TRB MiSeq 11	A011	*TRB* MiSeq 20	A020
TRB MiSeq 12	A012	*TRB* MiSeq 21	A021
TRB MiSeq 13	A013	*TRB* MiSeq 22	A022
TRB MiSeq 14	A014	*TRB* MiSeq 23	A023
TRB MiSeq 15	A015	*TRB* MiSeq 25	A025
TRB MiSeq 16	A016	*TRB* MiSeq 27	A027
Controls			
TRB POS (+) Qty. 3		NGS NEG (-) Qty. 3	



V-J Sequence Frequencies. The LymphoTrack bioinformatics software provides a stacked bar graph depicting the relative frequencies of the 200 most prevalent rearrangements sequenced and identified in the sample. To learn more about the LymphoTrack software, please refer to the LymphoTrack Bioinformatics Software section.

Ordering Information

Catalog #	Products	Quantity Components
7-225-0009	LymphoTrack® *TRB* Assay Kit A - MiSeq®	8 indices - 5 sequencing reactions each
7-225-0019	LymphoTrack® *TRB* Assay Panel - MiSeq®	24 indices - 5 sequencing reactions each
7-500-0009	LymphoTrack® Software - MiSeq®	1 CD complimentary with purchase
7-500-0008	LymphoTrack® MRD Software **NEW**	1 CD complimentary with purchase

LymphoTrack®

LymphoTrack Bioinformatics Software

Software Use

The LymphoTrack Bioinformatics Software package is provided with each LymphoTrack Assay to analyze raw FASTQ files for clonality analysis of single or multiple target data sets (*IGHV* Leader (Leader), *IGH* FR1, *IGH* FR2, *IGH* FR3, *IGK, TRG, TRB*). For data generated with LymphoTrack *IGHV* Leader or *IGH* FR1 Assays the software provides additional information, including the rate of somatic hypermutation (SHM) and whether a clone will be functional based upon the presence of a premature stop codon. The software can also predict whether an open reading frame would be in- or out-of-frame, so no external data analysis is required for sample interpretation.

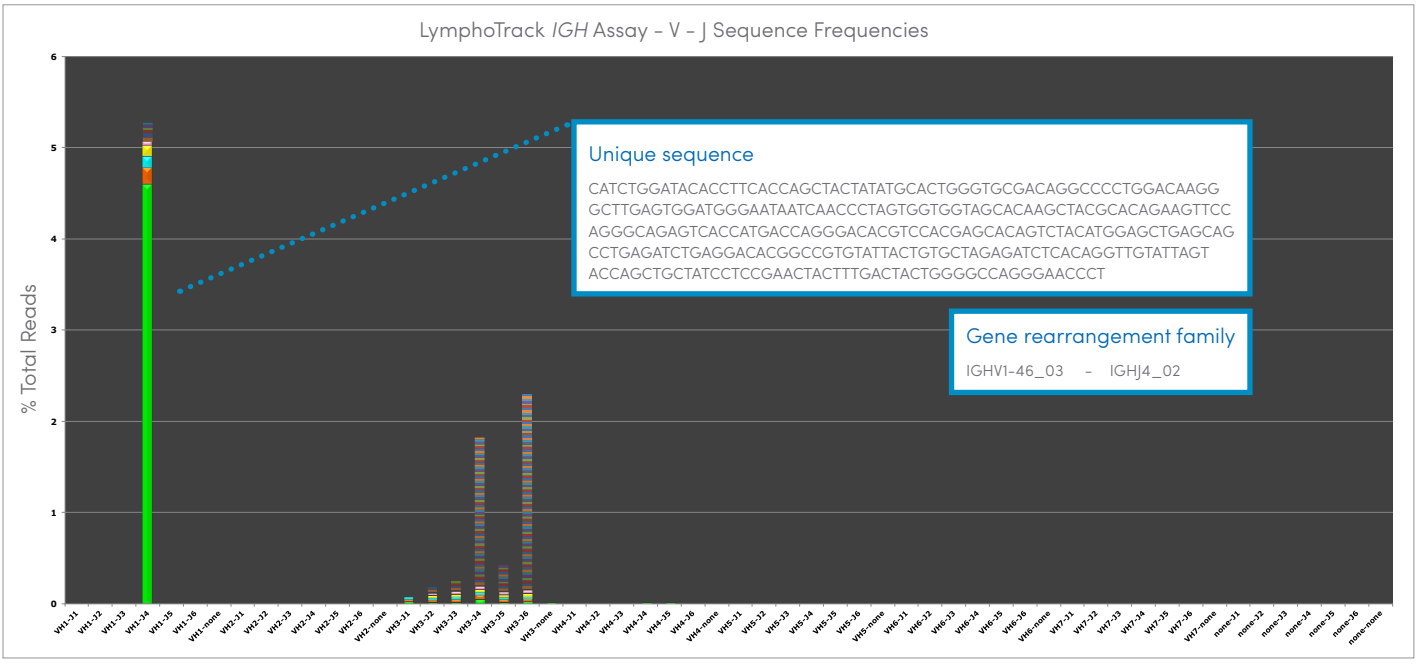
The provided software is composed of two distinct parts:

1. A bioinformatics Data Analysis Application
2. Microsoft Excel® Data Visualization *.xlsm* spreadsheets for each gene target

Minimum Software Requirements

- Processor: Intel Core 2 Duo or newer CPU recommended
- Hard Drive: At least 80 GB of free disk space is required; 250 GB recommended
- RAM: 4 GB required; 8 GB or more recommended
- Operating System: Windows 7 (64-bit) is required
- Java 7 for 64-bit operating systems or higher
- Microsoft Office Excel 2007, 2010, or 2013 with Macro support enabled is required for data visualization
- Microsoft Excel 2007/2010/2013 and Windows 7 configured for English (United States) language settings

Sequence Frequency Graph



The stacked bar graph depicts the top 200 sequencing reads for a sample. Each individual colored bar represents a unique population of cells. Different colors stacked at the same point on the x-axis represent unique sequences that utilize the same V and J gene families. The amplicons of these products vary in sequence and may also vary in product size.

Sequencing Summary

Using the merged read summary, along with the easy to follow flow charts in the accompanying LymphoTrack Assay instructions for use (IFU), interpretation is quick and easy.

1 Easy identification of specific types of gene rearrangements such as IGHV3-21.

Rank	Sequence	Length	Merge count	V-gene	J-gene	% Total reads	Cumulative %	Mutation rate partial V-gene (%)	In-frame (Y/N)	No stop codon (Y/N)	V-coverage
1	TTCTCGTGGTG	455	29603	IGHV4-59_08	IGHJ4_02	9.93	9.93	11.26	Y	Y	98.63
2	CTCGCCCTCCT	463	205	IGHV5-51_01	IGHJ4_02	0.07	9.99	0.00	Y	Y	99.66
3	GGTTTTCCTTG	484	201	IGHV3-7_01	IGHJ4_02	0.07	10.06	7.77	Y	Y	100.00
4	CTCGCCCTCCT	463	185	IGHV5-51_01	IGHJ5_02	0.06	10.12	6.08	Y	Y	99.32
5	CTCGCCCTCCT	469	170	IGHV5-51_01	IGHJ4_02	0.06	10.18	0.00	Y	Y	99.32
6	CTCGCCCTCCT	466	160	IGHV5-51_01	IGHJ4_02	0.05	10.23	0.00	Y	Y	99.66
7	CTGCTGCTGAC	460	159	IGHV2-5_10	IGHJ5_02	0.05	10.29	8.08	Y	Y	97.64
8	GGTTTTCCTTG	493	156	IGHV3-48_02	IGHJ6_02	0.05	10.34	3.72	Y	Y	98.99
9	CTCGCCCTCCT	334	153	IGHV5-51_02	IGHJ2_01	0.05	10.39	3.72	Y	N	27.70
10	CTCGCCCTCCT	334	152	IGHV5-51_02	IGHJ2_01	0.05	10.44	3.38	Y	N	26.01

2 Identification of clonal sequences for follow up tracking with LymphoTrack MRD Software.

3 SHM rate and indicators to determine whether a clone is productive. Only provided for *IGHV* Leader and *IGH* FR1.

1. Easy identification of specific types of gene rearrangements such as IGHV3-21.
2. Identification of clonal sequences for follow up tracking with LymphoTrack MRD Software.
3. SHM rate and indicators to determine whether a clone is productive. Only provided for *IGHV* Leader and *IGH* FR1.

The read summary provides sequences from a sample ranked from most abundant to least prevalent. The total read count for individual sequences is provided and no independent analysis is required to determine V and J gene families and predictions for SHM when analyzing data from LymphoTrack *IGHV* Leader or *IGH* FR1 Assays. Additionally, the software provides raw and merged data in which reads that are similar within 1-2 bp are combined. This simplifies the analysis and helps account for potential sequencing errors.

Ordering Information

Catalog #	Products	Quantity Components
7-500-0009	LymphoTrack® Software- MiSeq®	1 CD complimentary with kit purchase
7-500-0007	LymphoTrack® Software - PGM™	1 CD complimentary with kit purchase

LymphoTrack®

LymphoTrack Minimal Residual Disease (MRD) Software

Software Use

The LymphoTrack MRD Bioinformatics Software package is provided with each LymphoTrack Assay to enable monitoring of treatment response in ALL and other lymphoid malignancies. The exceptional sensitivity and precision of NGS-based MRD tracking can accelerate clinical trials and drug development. This MRD software is intended to detect the presence of clonotype sequences within the output files generated using the Invivoscribe LymphoTrack Assays and accompanying LymphoTrack bioinformatics software; it is not intended to define the significance of these findings. Once a specific rearrangement sequence (the clonotype) has been identified in a primary sample, the MRD software enables streamlined tracking of clonal populations at a sensitivity of 10^{-4}, or even lower limits provided sufficient DNA is tested. The MRD software can also be used for simultaneous objective tracking of bi-allelic sequences across multiple replicates for longitudinal subject tracking and in drug development studies.

The provided software is composed of three distinct parts:

1. A bioinformatics Data Analysis Application
2. A Project Planner that can be used to calculate confidence based on read depth, replicate count, and DNA input
3. Automated PDF report identifies the clonotype sequence if present and summarizes the degree of mismatches, calculates the read frequency and the degree of confidence when that clonotype is not present at 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} levels

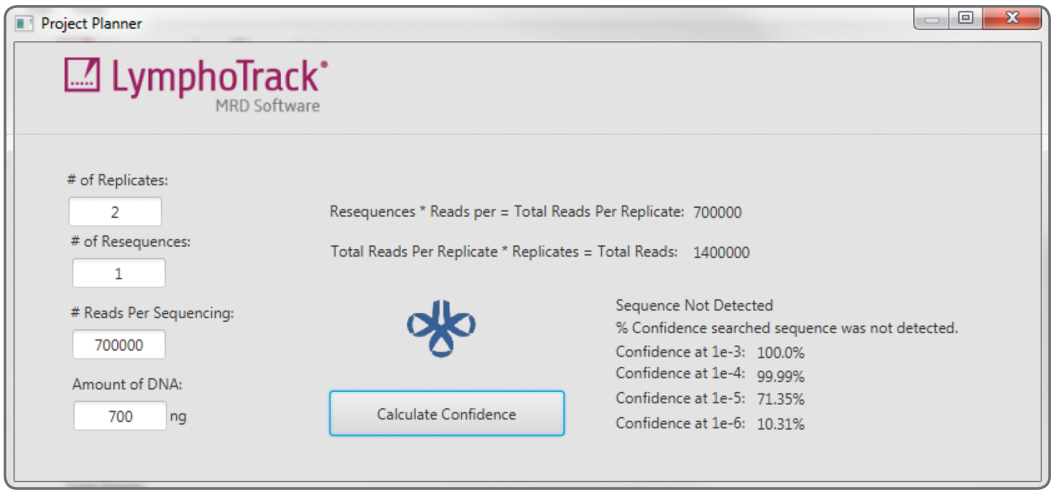
Minimum Software Requirements

- Processor: Intel Core 2 Duo or newer CPU recommended
- Hard Drive: At least 1 GB of free disk space is required; 2 GB recommended
- RAM: 4 GB required; 8 GB or more recommended
- Operating System: Windows 7 (64-bit) is required
- A CD-ROM drive*

* If a CD-ROM drive is not available, please contact us at: support@invivoscribe.com

MRD Project Planner

The Project Planner can be used to calculate the confidence of a true negative by adding replicate count, resequencing count, sequencing depth, and DNA input amount. The software assumes that the same sequencing depth and DNA input is used for each replicate.



LymphoTrack MRD Specimen Report

This report summarizes the overall call, i.e. if a clonotype was detected or not detected, the number of checked replicates, the total DNA input, total reads analyzed and the location of all output files. The values in the PDF report are also found in the generated text files.

Sequence: #1	Sequence Name: On Target Sequence	
Replicate	MRD Status	Reads in Replicate
1	Sequence Detected	437731

CATCTGGATACACCTTCACCAGCTACTATATGCACTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAATAATCAACCCTAGTGGTGGGCACAAGCTA
CGCACAGAAGTTCCAGGGCAGAGTCACCATGACCAGGGACACGTCCACGAGCACAGTCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATT
ACTGTGCTAGAGATCTCACAGGTTGTATTAGTACCAGCTGCTATCCTCCGAACTACTTTGACTACTGGGGCCAGGGAACCCT

Replicate Details	Read Count	Cumulative Read Count	Cumulative Read Frequency
Exact match	1281	1281	2.93×10^{-3}
1 Mismatch	0	1281	2.93×10^{-3}
2 Mismatch	0	1281	2.93×10^{-3}

Detection Limit	% Confidence
1×10^{-3}	N/A
1×10^{-4}	N/A
1×10^{-5}	N/A
1×10^{-6}	N/A

Sequence: #2	Sequence Name: Off Target Sequence	
Replicate	MRD Status	Reads in Replicate
1	Not Detected	437731

GCCTCTGGATTCACCTTCAGTAGGTACGACATGCACTGGGTCCGCCAAGGGACTGGAAAAGGTCTGGAGTGGGTCTCGGTTCCATCTCCAGAGAAAATGCCAAGAAC
TCCTTGGATCTTCAAATGAACAGCCTGAGAGCCGGGGACACGGCTGCATATTACTGTGGAAGAAGATAGCAGTAGTCGTTGAGTACTGGGGCCAGGGAACCCT

Replicate Details	Read Count	Cumulative Read Count	Cumulative Read Frequency
Exact match	0	0	0
1 Mismatch	0	0	0
2 Mismatch	0	0	0

Detection Limit	% Confidence
1×10^{-3}	100%
1×10^{-4}	99.89%
1×10^{-5}	28.79%
1×10^{-6}	2.0%

Ordering Information

Catalog #	Products	Quantity Components
7-500-0008	LymphoTrack® MRD Software**	1 CD complimentary with LymphoTrack kit purchase

** MRD Software can be used to track sequences generated using either LymphoTrack® Assays formatted for either the Illumina® or Thermo Fisher® NGS platforms.

Warranty and Liability

Invivoscribe Technologies, Inc. (Invivoscribe®) is committed to providing the highest quality products. Invivoscribe® warrants that the products meet or exceed the performance standards described in the Instructions For Use, as to products with such an insert. If a product is covered by product specifications and does not perform as specified, our policy is to replace the product or credit the full purchase price. No other warranties of any kind, expressed or implied, are provided by Invivoscribe®. Invivoscribe® liability shall not exceed the purchase price of the product. Invivoscribe® shall have no liability for direct, indirect, consequential or incidental damages arising from the use, results of use, or inability to use its products; product efficacy under purchaser controlled conditions in purchaser's laboratory must be established and continually monitored through purchaser defined and controlled processes including but not limited to testing of positive, negative, and blank controls every time a sample is tested. Ordering, acceptance, and use of product constitutes purchaser acceptance of sole responsibility for assuring product efficacy and purchaser agreement to the limitation of liability set forth in this paragraph.

NOTICE: The products in the section that follows are *in vitro* diagnostic products and are not available for sale or use within North America. Many of these products are covered by one or more of the following: European Patent Number 1549764, European Patent Number 2418287, European Patent Number 2460889, Japanese Patent Number 4708029, United States Patent 8859748, and related pending and future applications. All of these patents and applications are licensed exclusively to Invivoscribe®. Additional patents licensed to Invivoscribe covering some of these products apply elsewhere.

These products require nucleic acid amplification methods such as Polymerase Chain Reaction (PCR). No license under these patents to use amplification processes or enzymes is conveyed expressly or by implication to the purchaser by the purchase of these products.

Identiclone™ is a registered trademark of Invivoscribe®.

Gel and Capillary

CE IVD Assays

IdentiClone™

IdentiClone Assay kits are CE-marked *in vitro* diagnostic products*. These kits are intended for PCR-based detection of clonal gene rearrangements and translocations in patients with suspected lymphoproliferations, using gel or capillary electrophoresis methods.

These PCR-based tests include standardized Instructions For Use (IFUs) with interpretation guidelines describing the use of the kits' master mixes and controls. Master mixes are composed of a buffered magnesium chloride solution, deoxynucleotides, and multiple primers that target the gene segments of interest. Multiple primers are necessary to ensure a comprehensive testing approach that reliably identifies clonal rearrangements. These assay master mixes are complete with the exception of Taq DNA polymerase, which is not provided. A single thermocycler program and similar detection methods are used within each IdentiClone kit to improve consistency, reduce human error, and facilitate cross-training.

These assays are available in regular (33 reactions) and in MegaKit formats (330 reactions).

For more information, please visit www.invivoscribe.com

*NOTICE: IdentiClone Assays are *in vitro* diagnostic products and are not available for sale or use within North America. For more information regarding the research use only reagents, please see the Gel & Capillary Research Use Only Assays section.

IdentiClone™

IGH + *IGK* B-Cell Clonality Assays

Intended Use

The IdentiClone *IGH* + *IGK* B-Cell Clonality Assays are *in vitro* diagnostic products intended for PCR-based detection of clonal immunoglobulin heavy chain and kappa light chain gene rearrangements in patients with suspected lymphoproliferations.

Specifically, the IdentiClone *IGH* + *IGK* B-Cell Clonality Assays can be used to:

- Identify clonality in atypical lymphoproliferative disorders
- Support a differential diagnosis between reactive lesions and hematologic malignancies
- Assign presumptive lineage in mature monoclonal lymphoproliferative disorders
- Identify tumor-specific markers (*IGH* and *IGK* gene rearrangements) for post-treatment monitoring
- Monitor and evaluate disease recurrence

Summary and Explanation of the Test

The Invivoscribe CE-marked IdentiClone Assays represent a unique approach to PCR-based clonality testing. These standardized assays were carefully optimized, testing positive and negative control samples using multiplex master mixes. Assay development was followed by extensive validation including the testing of more than 400 clinical samples using Revised European/American Lymphoma (REAL) Classification. Testing was done at more than thirty prominent independent testing centers throughout Europe in a collaborative study known as the BIOMED-2 Concerted Action. Results from this BIOMED-2 study appear in *Leukemia*, a leading peer-reviewed journal[1].

These kits include 6 master mixes to test for rearrangements of both *IGH* and *IGK*. The *IGH* Tube A, B, and C master mixes target the framework 1, 2, 3 regions (respectively) within the variable (V_H) region, and the joining (J_H) region of the immunoglobulin heavy chain locus. The *IGK* Tube A master mix targets the variable (V_κ) and the joining (J_κ) region. *IGK* Tube B master mix targets kappa deleting element (Kde) rearrangements with the variable (V_κ) region and the intragenic J_κ-C_κ region. The resulting V_κ-Kde and J_κ-C_κ intron-Kde rearrangements are a result of unsuccessful rearrangements retained by the B cell. For best sensitivity, it is recommended to test suspect B-cell malignancies for both *IGH* and *IGK* (van Krieken, JHJM et al., Leukemia. 2007; 21:201 - 206). The included Specimen Control Size Ladder master mix targets multiple genes and generates a series of amplicons of approximately 100, 200, 300, 400, and 600 base pairs to ensure that the quality and quantity of input DNA is adequate to yield a valid result.

A single thermocycler program and similar detection methodologies are used with all of our Gene Clonality Assays. This improves consistency and facilitates cross-training on a broad range of different assays.

Performance Characteristics

Data from two independent studies that tested more than 300 patient samples of varying types suggests the diagnostic accuracy of selected IdentiClone tests to be 96%. In both peer-reviewed studies, there were no clear false-positive results generated using the IdentiClone tests, and there was a high level of precision[2]. The clinico-histopathological diagnosis correlated well with PCR results in a higher number of patients when compared with Southern Blot (SB) results, as seen below:

	PCR/SB concordance[1]:
IGH:	93% sensitivity / 92% specificity
IGK:	90% sensitivity / 90% specificity
IGL:	86% sensitivity / 92% specificity
TRB:	86% sensitivity / 98% specificity
TRG:	89% sensitivity / 94% specificity
TRD:	83% sensitivity / 95% specificity

PCR vs. SB analysis relative to histopathology and final diagnosis:

	PCR/SB concordance[2]:	PCR sensitivity:	SB sensitivity:
IGH + *IGK*:	85%	98%	39%
TRB:	85%	96%	35%

Reference

1. JJM van Dongen et al., *Leukemia* 17:2257-2317 (2003).
2. Y Sandberg et al., *J. Mol. Diag.* 7(4):495-503 (2005).

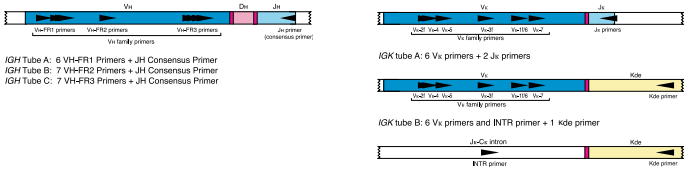


Figure Legend: Simple representation of the organization of a rearranged immunoglobulin heavy chain (*IGH*) gene on chromosome 14q32.33 and the immunoglobulin kappa light chain gene on chromosome 2p11.2. Black arrows represent the relative positions of primers that target the conserved framework regions (FR1-3) and the downstream consensus J_H gene segments for *IGH* and the V_κ, J_κ, INTR and Kde primers which are included in the *IGK* master mix tubes.

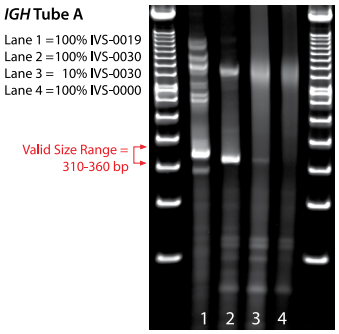
EuroClonality **This assay is based on the EuroClonality/BIOMED-2 Concerted Action BMH4-CT98-3936.**

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0030 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0019 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0007 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0000 Polyclonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	**Target**	**Units in Assay**	**Units in Assay MegaKit**
IGH Tube A	Framework 1 + JH	1 x 1500 µL tube	10 x 1500 µL tubes
IGH Tube B	Framework 2 + JH	1 x 1500 µL tube	10 x 1500 µL tubes
IGH Tube C	Framework 3 + JH	1 x 1500 µL tube	10 x 1500 µL tubes
IGK Tube A	Vκ-Jκ	1 x 1500 µL tube	10 x 1500 µL tubes
IGK Tube B	Vκ-Kde, Intron-Kde	1 x 1500 µL tube	10 x 1500 µL tubes
Specimen Control Size Ladder	Multiple Genes	1 x 1500 µL tube	10 x 1500 µL tubes

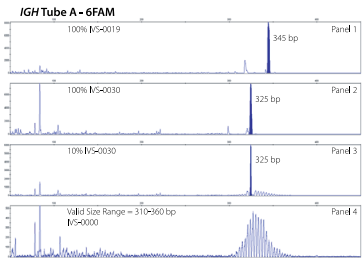
Polyacrylamide Gel Electrophoresis Detection

Heteroduplex analysis is performed to differentiate clonal and non-clonal PCR products. It involves heat denaturation of double-stranded DNA, followed by a snap chilling process to force DNA strands to quickly reanneal. In non-clonal populations, this process causes the majority of the single-stranded DNA to incorrectly bind to non-homologous strands, resulting in secondary structures and reducing the DNA's ability to migrate through a non-denaturing polyacrylamide gel. As a result, polyclonal products are frequently observed as smears at high molecular weights. In clonal populations, after heteroduplex analysis is performed, most denatured single-strand PCR products will reanneal correctly with homologous strands of DNA (reforming homoduplexes) allowing them to easily migrate through the polyacrylamide gel as a single band. Therefore, heteroduplex analysis is key for analyzing PCR products visualized using gel detection methods, as it increases the separation between clonal and polyclonal products.



Capillary Electrophoresis Detection (ABI)

Differential fluorescence detection, such as ABI fluorescence detection, is commonly used to resolve different-sized amplicon products using a capillary electrophoresis instrument. Primers can be conjugated with several different fluorescent dyes (fluorophores), so that they produce different emission spectra upon excitation by a laser in the capillary electrophoresis instrument. In this manner, different fluorescent dyes can correspond to different targeted regions. This detection system results in excellent sensitivity, single nucleotide resolution, differential product detection, and relative quantification. In addition, differential detection allows accurate, reproducible and objective interpretation of primer-specific products. Inter-assay and intra-assay reproducibility in size determination using capillary electrophoresis is approximately 1 to 4 nucleotides.



Ordering Information

Catalog #	Products	Quantity
9-100-0010	IdentiClone™ *IGH* + *IGK* B-Cell Clonality Assay - Gel Detection	33 reactions
9-100-0020	IdentiClone™ *IGH* + *IGK* B-Cell Clonality Assay MegaKit - Gel Detection	330 reactions
9-100-0031	IdentiClone™ *IGH* + *IGK* B-Cell Clonality Assay - ABI Fluorescence Detection	33 reactions
9-100-0041	IdentiClone™ *IGH* + *IGK* B-Cell Clonality Assay MegaKit - ABI Fluorescence Detection	330 reactions

These are *in vitro* diagnostic products, and are not available for sale or use within North America.

IdentiClone™

IGH Gene Clonality Assays

Intended Use

The IdentiClone *IGH* Gene Clonality Assays are *in vitro* diagnostic products intended for PCR-based detection of clonal immunoglobulin heavy chain gene rearrangements in patients with suspected lymphoproliferations.

Specifically, the IdentiClone *IGH* Gene Clonality Assays can be used to:

- Identify clonality in atypical lymphoproliferative disorders
- Support a differential diagnosis between reactive lesions and hematologic malignancies
- Assign presumptive lineage in mature monoclonal lymphoproliferative disorders
- Identify tumor-specific markers (*IGH* gene rearrangements) for post-treatment monitoring
- Monitor and evaluate disease recurrence

Summary and Explanation of the Test

The Invivoscribe CE-marked IdentiClone Assays represent a unique approach to PCR-based clonality testing. These standardized assays were carefully optimized, testing positive and negative control samples using multiplex master mixes. Assay development was followed by extensive validation including the testing of more than 400 clinical samples using Revised European/American Lymphoma (REAL) Classification. Testing was done at more than thirty prominent independent testing centers throughout Europe in a collaborative study known as the BIOMED-2 Concerted Action. Results from this BIOMED-2 study appear in *Leukemia*, a leading peer-reviewed journal[1].

These test kits include 6 master mixes. The *IGH* Tube A, B, and C master mixes target the framework 1, 2, and 3 regions (respectively) within the variable (VH) region and the joining (JH) region of the immunoglobulin heavy chain locus. The *IGH* Tube D and E master mixes target the diversity and joining regions. The Specimen Control Size Ladder master mix targets multiple genes and generates a series of amplicons of approximately 100, 200, 300, 400, and 600 base pairs to ensure that the quality and quantity of input DNA is adequate to yield a valid result. A single thermocycler program and similar detection methodologies are used with all of our gene clonality assays. This improves consistency and facilitates cross-training on a broad range of different assays.

Performance Characteristics

Data from two independent studies that tested more than 300 patient samples of varying types suggests the diagnostic accuracy of selected IdentiClone tests to be 96%. In both peer-reviewed studies, there were no clear false-positive results generated using the IdentiClone tests, and there was a high level of precision[2]. The clinico-histopathological diagnosis correlated well with PCR results in a higher number of patients when compared with Southern Blot (SB) results, as seen below:

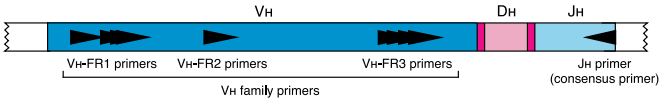
	PCR/SB concordance[1]:
IGH:	93% sensitivity / 92% specificity
IGK:	90% sensitivity / 90% specificity
IGL:	86% sensitivity / 92% specificity
TRB:	86% sensitivity / 98% specificity
TRG:	89% sensitivity / 94% specificity
TRD:	83% sensitivity / 95% specificity

PCR vs. SB analysis relative to histopathology and final diagnosis:

	PCR/SB concordance[2]:	PCR sensitivity:	SB sensitivity:
IGH + *IGK*:	85%	98%	39%
TRB:	85%	96%	35%

Reference

1. JJM van Dongen et al., *Leukemia* 17:2257-2317 (2003).
2. Y Sandberg et al., *J. Mol. Diag.* 7(4):495-503 (2005).



Tube A: 6 V_H -FR1 Primers + J_H Consensus Primer
Tube B: 7 V_H -FR2 Primers + J_H Consensus Primer
Tube C: 7 V_H -FR3 Primers + J_H Consensus Primer

Tube D: 6 D_H Primers + J_H Consensus Primer
Tube E: D_H 7 Primer + J_H Consensus Primer

Figure Legend: Simple representation of the organization of a rearranged immunoglobulin heavy chain gene on chromosome 14q32.33. Black arrows represent the relative positions of primers that target the conserved framework (FR1-3) and diversity (DH1-7) regions, and the downstream consensus JH gene segments. The amplicon products generated from each of these regions can be differentially detected when fluorescent primer sets are used with capillary electrophoresis instruments that employ differential fluorescence detection.

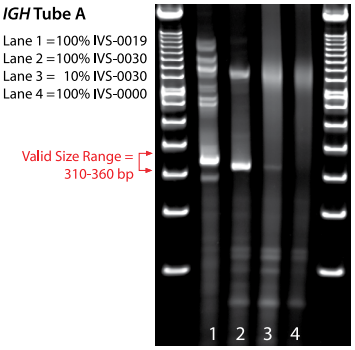
EuroClonality This assay is based on the **EuroClonality/BIOMED-2** Concerted Action BMH4-CT98-3936.

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0030 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0019 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0024 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0008 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0000 Polyclonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	**Target**	**Units in Assay**	**Units in Assay MegaKit**
IGH Tube A	Framework 1 + JH	1 x 1500 µL tube	10 x 1500 µL tubes
IGH Tube B	Framework 2 + JH	1 x 1500 µL tube	10 x 1500 µL tubes
IGH Tube C	Framework 3 + JH	1 x 1500 µL tube	10 x 1500 µL tubes
IGH Tube D	DH1-6 + JH	1 x 1500 µL tube	10 x 1500 µL tubes
IGH Tube E	DH7 + JH	1 x 1500 µL tube	10 x 1500 µL tubes
Specimen Control Size Ladder	Multiple Genes	1 x 1500 µL tube	10 x 1500 µL tubes

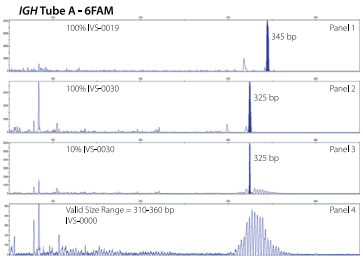
Polyacrylamide Gel Electrophoresis Detection

Heteroduplex analysis is performed to differentiate clonal and non-clonal PCR products and involves heat denaturation of double-stranded DNA, followed by a snap chilling process to force DNA strands to quickly reanneal. In non-clonal populations, this process causes a majority of the single-stranded DNA to incorrectly bind to non-homologous strands resulting in secondary structures and reducing the DNA's ability to migrate through a non-denaturing polyacrylamide gel. As a result, polyclonal products are frequently observed as smears at high molecular weights. In clonal populations, after heteroduplex analysis is performed, most denatured single-strand PCR products will reanneal correctly with homologous strands of DNA (reforming homoduplexes) allowing them to easily migrate through the polyacrylamide gel as a single band. Therefore, heteroduplex analysis is key for analyzing PCR products visualized using gel detection methods as it increases the separation between clonal and polyclonal products.



Capillary Electrophoresis Detection (ABI)

Differential fluorescence detection, such as ABI fluorescence detection, is commonly used to resolve different-sized amplicon products using a capillary electrophoresis instrument. Primers can be conjugated with several different fluorescent dyes (fluorophores), so that they produce different emission spectra upon excitation by a laser in the capillary electrophoresis instrument. In this manner, different fluorescent dyes can correspond to different targeted regions. This detection system results in excellent sensitivity, single nucleotide resolution, differential product detection, and relative quantification. In addition, differential detection allows accurate, reproducible and objective interpretation of primer-specific products. Inter-assay and intra-assay reproducibility in size determination using capillary electrophoresis is approximately 1 to 4 nucleotides.



Ordering Information

Catalog #	Products	Quantity
9-101-0020	IdentiClone™ *IGH* Gene Clonality Assay - Gel Detection	33 reactions
9-101-0040	IdentiClone™ *IGH* Gene Clonality Assay MegaKit - Gel Detection	330 reactions
9-101-0061	IdentiClone™ *IGH* Gene Clonality Assay - ABI Fluorescence Detection	33 reactions
9-101-0081	IdentiClone™ *IGH* Gene Clonality Assay MegaKit - ABI Fluorescence Detection	330 reactions

These are *in vitro* diagnostic products, and are not available for sale or use within North America.

IdentiClone™

IGK Gene Clonality Assays

Intended Use

The IdentiClone *IGK* Gene Clonality Assays are *in vitro* diagnostic products intended for PCR-based detection of clonal immunoglobulin kappa light chain gene rearrangements in patients with suspected lymphoproliferations.

Specifically, the IdentiClone *IGK* Gene Clonality Assays can be used to:

- Identify clonality in atypical lymphoproliferative disorders
- Support a differential diagnosis between reactive lesions and hematologic malignancies
- Assign presumptive lineage in mature monoclonal lymphoproliferative disorders
- Identify tumor-specific markers (*IGK* and *IGK*-Kde rearrangements) for post-treatment monitoring
- Monitor and evaluate disease recurrence

Summary and Explanation of the Test

The Invivoscribe CE-marked IdentiClone Assays represent a unique approach to PCR-based clonality testing. These standardized assays were carefully optimized testing positive and negative control samples using multiplex master mixes. Assay development was followed by extensive validation including the testing of more than 400 clinical samples using Revised European/American Lymphoma (REAL) Classification. Testing was done at more than thirty prominent independent testing centers throughout Europe in a collaborative study known as the BIOMED-2 Concerted Action. Results from this BIOMED-2 study appear in *Leukemia*, a leading peer-reviewed journal[1].

These test kits include 3 master mixes. The *IGK* Tube A master mix targets the variable (Vκ) and the joining (Jκ) regions of the immunoglobulin kappa light chain locus, whereas the *IGK* Tube B master mix targets kappa deleting element (Kde) rearrangements with the Vκ regions and the intragenic Jκ-Cκ regions. The Vκ-Kde and Jκ-Cκ intron-Kde rearrangements are a result of unsuccessful rearrangements retained by the B cell. The third master mix, the Specimen Control Size Ladder, targets multiple genes and generates a series of amplicons of approximately 100, 200, 300, 400, and 600 base pairs to ensure that the quality and quantity of input DNA is adequate to yield a valid result. A single thermocycler program and similar detection methodologies are used with all of our gene clonality assays. This improves consistency and facilitates cross-training.

Performance Characteristics

Data from two independent studies that tested more than 300 patient samples of varying types suggests the diagnostic accuracy of selected IdentiClone tests to be 96%. In both peer-reviewed studies, there were no clear false-positive results generated using the IdentiClone tests, and there was a high level of precision[2]. The clinico-histopathological diagnosis correlated well with PCR results in a higher number of patients when compared with Southern Blot (SB) results, as seen below:

	PCR/SB concordance[1]:
IGH:	93% sensitivity / 92% specificity
IGK:	90% sensitivity / 90% specificity
IGL:	86% sensitivity / 92% specificity
TRB:	86% sensitivity / 98% specificity
TRG:	89% sensitivity / 94% specificity
TRD:	83% sensitivity / 95% specificity

PCR vs. SB analysis relative to histopathology and final diagnosis:

	PCR/SB concordance[2]:	PCR sensitivity:	SB sensitivity:
IGH + *IGK*:	85%	98%	39%
TRB:	85%	96%	35%

Reference

1. JJM van Dongen et al., *Leukemia* 17:2257-2317 (2003).
2. Y Sandberg et al., *J. Mol. Diag.* 7(4):495-503 (2005).

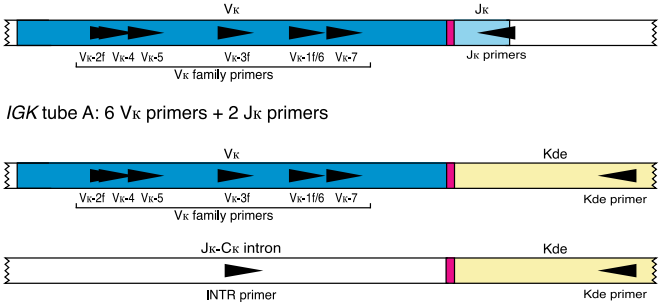


Figure Legend: Schematic diagram of the immunoglobulin kappa light chain gene complex on chromosome 2p11.2. Shown are the relative positions and orientations for the Vκ-Jκ, and Kde primers, which are included in the *IGK* master mix tubes.

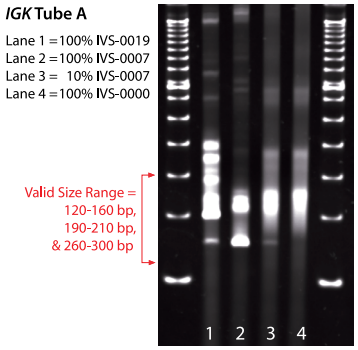
EuroClonality This assay is based on the **EuroClonality/BIOMED-2** Concerted Action BMH4-CT98-3936.

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0007 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0000 Polyclonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	**Target**	**Units in Assay**	**Units in Assay MegaKit**
IGK Tube A	Vκ – Jκ	1 x 1500 µL tube	10 x 1500 µL tubes
IGK Tube B	Vκ-Kde, Intron-Kde	1 x 1500 µL tube	10 x 1500 µL tubes
Specimen Control Size Ladder	Multiple Genes	1 x 1500 µL tube	10 x 1500 µL tubes

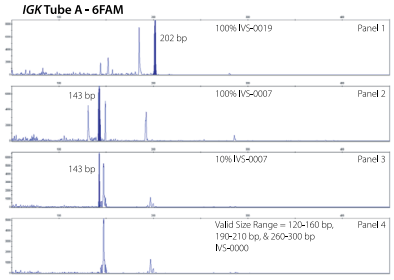
Polyacrylamide Gel Electrophoresis Detection

Heteroduplex analysis is performed to differentiate clonal and non-clonal PCR products. It involves heat denaturation of double-stranded DNA, followed by a snap chilling process to force DNA strands to quickly reanneal. In non-clonal populations, this process causes the majority of the single-stranded DNA to incorrectly bind to non-homologous strands, resulting in secondary structures and reducing the DNA's ability to migrate through a non-denaturing polyacrylamide gel. As a result, polyclonal products are frequently observed as smears at high molecular weights. In clonal populations, after heteroduplex analysis is performed, most denatured single-strand PCR products will reanneal correctly with homologous strands of DNA (reforming homoduplexes), allowing them to easily migrate through the polyacrylamide gel as a single band. Therefore, heteroduplex analysis is key for analyzing PCR products visualized using gel detection methods, as it increases the separation between clonal and polyclonal products.



Capillary Electrophoresis Detection (ABI)

Differential fluorescence detection, such as ABI fluorescence detection, is commonly used to resolve different-sized amplicon products using a capillary electrophoresis instrument. Primers can be conjugated with several different fluorescent dyes (fluorophores), so that they produce different emission spectra upon excitation by a laser in the capillary electrophoresis instrument. In this manner, different fluorescent dyes can correspond to different targeted regions. This detection system results in excellent sensitivity, single nucleotide resolution, differential product detection, and relative quantification. In addition, differential detection allows accurate, reproducible and objective interpretation of primer-specific products. Inter-assay and intra-assay reproducibility in size determination using capillary electrophoresis is approximately 1 to 4 nucleotides.



Ordering Information

Catalog #	Products	Quantity
9-102-0020	IdentiClone™ *IGK* Gene Clonality Assay - Gel Detection	33 reactions
9-102-0030	IdentiClone™ *IGK* Gene Clonality Assay MegaKit - Gel Detection	330 reactions
9-102-0021	IdentiClone™ *IGK* Gene Clonality Assay - ABI Fluorescence Detection	33 reactions
9-102-0031	IdentiClone™ *IGK* Gene Clonality Assay MegaKit - ABI Fluorescence Detection	330 reactions

These are *in vitro* diagnostic products, and are not available for sale or use within North America.

IdentiClone™

IGL Gene Clonality Assays

Intended Use

The IdentiClone *IGL* Gene Clonality Assays are *in vitro* diagnostic products intended for PCR-based detection of clonal immunoglobulin lambda light chain gene rearrangements in patients with suspected lymphoproliferations.

Specifically, the IdentiClone *IGL* Gene Clonality Assays can be used to:

- Identify clonality in atypical lymphoproliferative disorders
- Support a differential diagnosis between reactive lesions and hematologic malignancies
- Assign presumptive lineage in mature monoclonal lymphoproliferative disorders
- Identify tumor-specific markers (*IGL* gene rearrangements) for post-treatment monitoring
- Monitor and evaluate disease recurrence

Summary and Explanation of the Test

The Invivoscribe CE-marked IdentiClone Assays represent a unique approach to PCR-based clonality testing. These standardized assays were carefully optimized testing positive and negative control samples using multiplex master mixes. Assay development was followed by extensive validation including the testing of more than 400 clinical samples using Revised European/American Lymphoma (REAL) Classification. Testing was done at more than thirty prominent independent testing centers throughout Europe in a collaborative study known as the BIOMED-2 Concerted Action. Results from this BIOMED-2 study appear in *Leukemia,* a leading peer-reviewed journal[1].

These test kits include 2 master mixes. The *IGL* Tube master mix targets the variable (Vλ) region and the joining (Jλ) region of the immunoglobulin lambda light chain gene locus (*IGL*). The Specimen Control Size Ladder targets multiple genes and generates a series of amplicons of approximately 100, 200, 300, 400, and 600 base pairs to ensure that the quality and quantity of input DNA is adequate to yield a valid result. A single thermocycler program and similar detection methodologies are used with all of our gene clonality assays. This improves consistency and facilitates cross-training.

Performance Characteristics

Data from two independent studies that tested more than 300 patient samples of varying types suggests the diagnostic accuracy of selected IdentiClone tests to be 96%. In both peer-reviewed studies, there were no clear false-positive results generated using the IdentiClone tests, and there was a high level of precision[2]. The clinico-histopathological diagnosis correlated well with PCR results in a higher number of patients when compared with Southern Blot (SB) results, as seen below:

	PCR/SB concordance[1]:
IGH:	93% sensitivity / 92% specificity
IGK:	90% sensitivity / 90% specificity
IGL:	86% sensitivity / 92% specificity
TRB:	86% sensitivity / 98% specificity
TRG:	89% sensitivity / 94% specificity
TRD:	83% sensitivity / 95% specificity

PCR vs. SB analysis relative to histopathology and final diagnosis:

	PCR/SB concordance[2]:	PCR sensitivity:	SB sensitivity:
IGH + *IGK*:	85%	98%	39%
TRB:	85%	96%	35%

Reference

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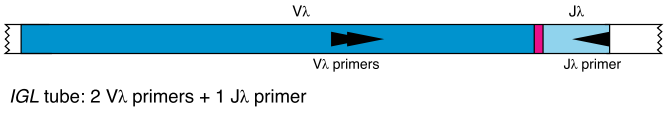


IGL tube: 2 Vλ primers + 1 Jλ primer

Figure Legend: Schematic diagram of the immunoglobulin lambda light chain gene complex on chromosome 22q11.2. Shown are the relative positions and orientations for the Vλ and Jλ primers, which are included in the *IGL* master mix tube. The two Vλ primers only target Vλ1, 2, and 3 because these three V families cover approximately 70% of rearrangeable Vλ gene segments, and approximately 90% of all *IGL* gene rearrangements involve these three families. Similarly, the single Jλ primer only targets Jλ1, 2, and 3 because these three J segments are involved in 98% of all *IGL* gene rearrangements.

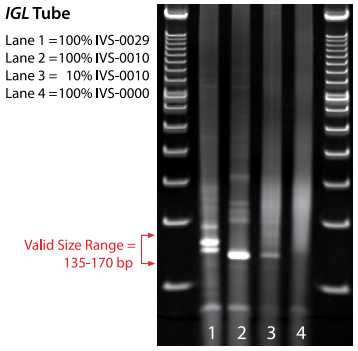
EuroClonality This assay is based on the **EuroClonality/BIOMED-2** Concerted Action BMH4-CT98-3936.

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0010 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0029 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0000 Polyclonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	**Target**	**Units in Assay**	**Units in Assay MegaKit**
IGL Tube	Vλ -Jλ	1 x 1500 µL tube	10 x 1500 µL tubes
Specimen Control Size Ladder	Multiple Genes	1 x 1500 µL tube	10 x 1500 µL tubes

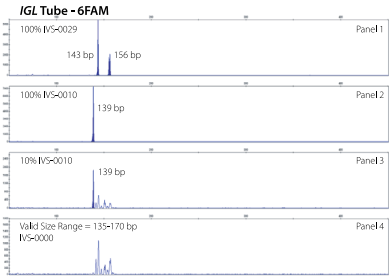
Polyacrylamide Gel Electrophoresis Detection

Heteroduplex analysis is performed to differentiate clonal and non-clonal PCR products It involves heat denaturation of double-stranded DNA, followed by a snap chilling process to force DNA strands to quickly reanneal. In non-clonal populations, this process causes a majority of the single-stranded DNA to incorrectly bind to non-homologous strands, resulting in secondary structures and reducing the DNA's ability to migrate through a non-denaturing polyacrylamide gel. As a result, polyclonal products are frequently observed as smears at high molecular weights. In clonal populations, after heteroduplex analysis is performed, most denatured single-strand PCR products will reanneal correctly with homologous strands of DNA (reforming homoduplexes), allowing them to easily migrate through the polyacrylamide gel as a single band. Therefore, heteroduplex analysis is key for analyzing PCR products visualized using gel detection methods, as it increases the separation between clonal and polyclonal products.



Capillary Electrophoresis Detection (ABI)

Differential fluorescence detection, such as ABI fluorescence detection, is commonly used to resolve different-sized amplicon products using a capillary electrophoresis instrument. Primers can be conjugated with several different fluorescent dyes (fluorophores), so that they produce different emission spectra upon excitation by a laser in the capillary electrophoresis instrument. In this manner, different fluorescent dyes can correspond to different targeted regions. This detection system results in excellent sensitivity, single nucleotide resolution, differential product detection, and relative quantification. In addition, differential detection allows accurate, reproducible and objective interpretation of primer-specific products. Inter-assay and intra-assay reproducibility in size determination using capillary electrophoresis is approximately 1 to 4 nucleotides.



Ordering Information

Catalog #	Products	Quantity
9-103-0010	IdentiClone™ *IGL* Gene Clonality Assay - Gel Detection	33 reactions
9-103-0020	IdentiClone™ *IGL* Gene Clonality Assay MegaKit - Gel Detection	330 reactions
9-103-0011	IdentiClone™ *IGL* Gene Clonality Assay - ABI Fluorescence Detection	33 reactions
9-103-0021	IdentiClone™ *IGL* Gene Clonality Assay MegaKit - ABI Fluorescence Detection	330 reactions

These are *in vitro* diagnostic products, and are not available for sale or use within North America.

IdentiClone™

TCRB + *TCRG* T-Cell Clonality Assays

Intended Use

The IdentiClone *TCRB* + *TCRG* T-Cell Clonality Assays are *in vitro* diagnostic products intended for PCR-based detection of clonal T-cell receptor beta and gamma chain gene rearrangements in patients with suspected lymphoproliferations.

Specifically, the IdentiClone *TCRB* + *TCRG* T-Cell Clonality Assays can be used to:

- Identify clonality in suspected lymphoproliferations
- Support a differential diagnosis between reactive lesions and T-cell and some immature B-cell malignancies
- Determine lineage involvement in mature lymphoproliferative disorders
- Monitor and evaluate disease recurrence

Summary and Explanation of the Test

The Invivoscribe CE-marked IdentiClone Assays represent a unique approach to PCR-based clonality testing. These standardized assays were carefully optimized testing positive and negative control samples using multiplex master mixes. Assay development was followed by extensive validation including the testing of more than 400 clinical samples using Revised European/American Lymphoma (REAL) Classification. Testing was done at more than thirty prominent independent testing centers throughout Europe in a collaborative study known as the BIOMED-2 Concerted Action. Results from this BIOMED-2 study appear in *Leukemia*, a leading peer-reviewed journal[1].

These kits include 6 master mixes to test for rearrangements of both *TRB* (formerly known as *TCRB*) and *TRG* (formerly known as *TCRG*). *TCRB* Tubes A and B target framework regions within the variable region, and the joining region of the *TCR* beta chain locus. *TCRB* Tube C targets the diversity and joining regions of the TCR beta chain locus. *TCRG* Tube A contains primers that target the Vγ1-8 + Vγ10 genes and Jγ1.1, Jγ1.3, Jγ2.1, and Jγ2.3 genes (also known as JγP1, Jγ1, JγP2, and Jγ2 respectively). *TCRG* Tube B contains primers that target the Vγ9 + Vγ11 genes and Jγ1.1, Jγ1.3, Jγ2.1, and Jγ2.3 genes. For best sensitivity it is recommended to test suspect T-cell malignancies for both *TRG* and *TRB* (van Krieken, JHJM. et al., Leukemia. 2007; 21:201- 206). The Specimen Control Size Ladder master mix included targets multiple genes and generates a series of amplicons of approximately 100, 200, 300, 400, and 600 base pairs to ensure that the quality and quantity of input DNA is adequate to yield a valid result. A single thermocycler program and similar detection methodologies are used with all of our gene clonality assays. This improves consistency and facilitates cross-training on a broad range of different assays.

Performance Characteristics

Data from two independent studies that tested more than 300 patient samples of varying types suggests the diagnostic accuracy of selected IdentiClone tests to be 96%. In both peer-reviewed studies, there were no clear false-positive results generated using the IdentiClone tests, and there was a high level of precision[2]. The clinico-histopathological diagnosis correlated well with PCR results in a higher number of patients when compared with Southern Blot (SB) results, as seen below:

	PCR/SB concordance[1]:
IGH:	93% sensitivity / 92% specificity
IGK:	90% sensitivity / 90% specificity
IGL:	86% sensitivity / 92% specificity
TRB:	86% sensitivity / 98% specificity
TRG:	89% sensitivity / 94% specificity
TRD:	83% sensitivity / 95% specificity

PCR vs. SB analysis relative to histopathology and final diagnosis:

	PCR/SB concordance[2]:	PCR sensitivity:	SB sensitivity:
IGH + *IGK*:	85%	98%	39%
TRB:	85%	96%	35%

Reference

1. JJM van Dongen et al., *Leukemia* 17:2257-2317 (2003).
2. Y Sandberg et al., *J. Mol. Diag.* 7(4):495-503 (2005).

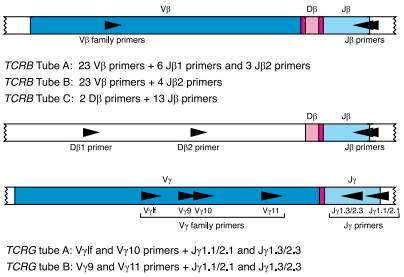


Figure Legend: Simplified diagram of a representative rearranged T-cell receptor beta gene and the T-cell receptor gamma gene showing the approximate placement of the upstream and downstream DNA primers. The numbers of primers and their specificity are listed for master mix *TCRB* Tubes A, B, and C and TCRG Tubes A and B. (The Vγ1f primer is a consensus primer that targets Vγ1 through Vγ8).

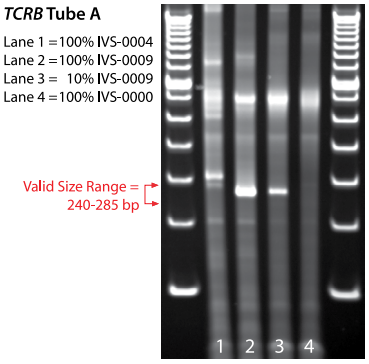
EuroClonality **This assay is based on the EuroClonality/BIOMED-2 Concerted Action BMH4-CT98-3936.**

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0009 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0004 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0021 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0000 Polyclonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	**Target**	**Units in Assay**	**Units in Assay MegaKit**
TCRB Tube A	Multiple Vβ + Jβ1/2	1 x 1500 µL tube	10 x 1500 µL tubes
TCRB Tube B	Multiple Vβ + Jβ2	1 x 1500 µL tube	10 x 1500 µL tubes
TCRB Tube C	Multiple Dβ + Jβ1/2	1 x 1500 µL tube	10 x 1500 µL tubes
TCRG Tube A	Vγβ1-8 + Vγ10 + Jγ	1 x 1500 µL tube	10 x 1500 µL tubes
TCRG Tube B	Vγ9 + Vγ11 + Jγ	1 x 1500 µL tube	10 x 1500 µL tubes
Specimen Control Size Ladder	Multiple Genes	1 x 1500 µL tube	10 x 1500 µL tubes

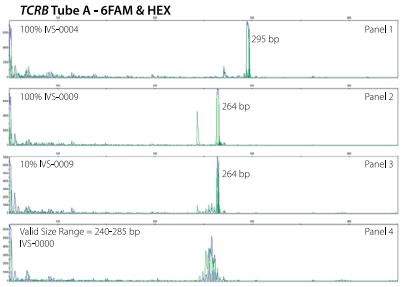
Polyacrylamide Gel Electrophoresis Detection

Heteroduplex analysis is performed to differentiate clonal and non-clonal PCR products. It involves heat denaturation of double-stranded DNA, followed by a snap chilling process to force DNA strands to quickly reanneal. In non-clonal populations, this process causes a majority of the single-stranded DNA to incorrectly bind to non-homologous strands, resulting in secondary structures and reducing the DNA's ability to migrate through a non-denaturing polyacrylamide gel. As a result, polyclonal products are frequently observed as smears at high molecular weights. In clonal populations, after heteroduplex analysis is performed, most denatured single-strand PCR products will reanneal correctly with homologous strands of DNA (reforming homoduplexes), allowing them to easily migrate through the polyacrylamide gel as a single band. Therefore, heteroduplex analysis is key for analyzing PCR products visualized using gel detection methods, as it increases the separation between clonal and polyclonal products.



Capillary Electrophoresis Detection (ABI)

Differential fluorescence detection, such as ABI fluorescence detection, is commonly used to resolve different-sized amplicon products using a capillary electrophoresis instrument. Primers can be conjugated with several different fluorescent dyes (fluorophores), so that they produce different emission spectra upon excitation by a laser in the capillary electrophoresis instrument. In this manner, different fluorescent dyes can correspond to different targeted regions. This detection system results in excellent sensitivity, single nucleotide resolution, differential product detection, and relative quantification. In addition, differential detection allows accurate, reproducible and objective interpretation of primer-specific products. Inter-assay and intra-assay reproducibility in size determination using capillary electrophoresis is approximately 1 to 4 nucleotides.



Ordering Information

Catalog #	Products	Quantity
9-200-0010	IdentiClone™ *TCRB* + *TCRG* T-Cell Clonality Assay - Gel Detection	33 reactions
9-200-0020	IdentiClone™ *TCRB* + *TCRG* T-Cell Clonality Assay MegaKit - Gel Detection	330 reactions
9-200-0011	IdentiClone™ *TCRB* + *TCRG* T-Cell Clonality Assay - ABI Fluorescence Detection	33 reactions
9-200-0021	IdentiClone™ *TCRB* + *TCRG* T-Cell Clonality Assay MegaKit - ABI Fluorescence Detection	330 reactions

These are *in vitro* diagnostic products, and are not available for sale or use within North America.

IdentiClone™

TCRB Gene Clonality Assays

Intended Use

The IdentiClone *TCRB* Gene Clonality Assays are *in vitro* diagnostic products intended for PCR-based detection of clonal T-cell receptor beta chain gene rearrangements in patients with suspected lymphoproliferations.

Specifically, the IdentiClone *TCRB* Gene Clonality Assay can be used to:

- Identify clonality in suspected lymphoproliferations
- Support a differential diagnosis between reactive lesions and T-cell and some immature B-cell malignancies
- Determine lineage involvement in mature lymphoproliferative disorders
- Monitor and evaluate disease recurrence

Summary and Explanation of the Test

The Invivoscribe CE-marked IdentiClone Assays represent a unique approach to PCR-based clonality testing. These standardized assays were carefully optimized testing positive and negative control samples using multiplex master mixes. Assay development was followed by extensive validation including the testing of more than 400 clinical samples using Revised European/American Lymphoma (REAL) Classification. Testing was done at more than thirty prominent independent testing centers throughout Europe in a collaborative study known as the BIOMED-2 Concerted Action. Results from this BIOMED-2 study appear in *Leukemia*, a leading peer-reviewed journal[1].

These kits include 4 master mixes. *TCRB* Tubes A and B target framework regions within the variable region, and the joining region (Vβ) of the *TCR* beta chain locus. *TCRB* Tube C targets the diversity and joining (Jβ) regions of the TCR beta chain locus. The Specimen Control Size Ladder master mix included targets multiple genes and generates a series of amplicons of approximately 100, 200, 300, 400, and 600 base pairs to ensure that the quality and quantity of input DNA is adequate to yield a valid result. A single thermocycler program and similar detection methodologies are used with all of our Gene Clonality Assays. This improves consistency and facilitates cross-training on a broad range of different assays.

Performance Characteristics

Data from two independent studies that tested more than 300 patient samples of varying types suggests the diagnostic accuracy of selected IdentiClone tests to be 96%. In both peer-reviewed studies, there were no clear false-positive results generated using the IdentiClone tests, and there was a high level of precision[2]. The clinico-histopathological diagnosis correlated well with PCR results in a higher number of patients when compared with Southern Blot (SB) results, as seen below:

	PCR/SB concordance[1]:
IGH:	93% sensitivity / 92% specificity
IGK:	90% sensitivity / 90% specificity
IGL:	86% sensitivity / 92% specificity
TRB:	86% sensitivity / 98% specificity
TRG:	89% sensitivity / 94% specificity
TRD:	83% sensitivity / 95% specificity

PCR vs. SB analysis relative to histopathology and final diagnosis:

	PCR/SB concordance[2]:	PCR sensitivity:	SB sensitivity:
IGH + *IGK*:	85%	98%	39%
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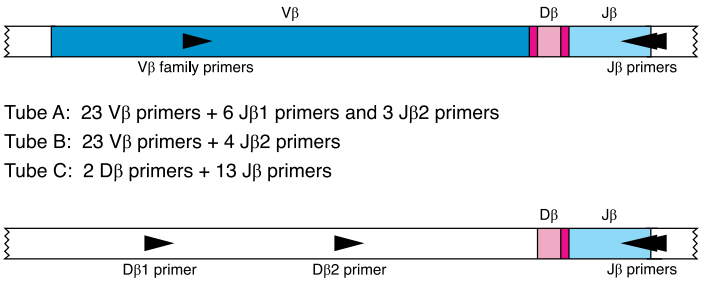


Figure Legend: Simplified diagram of a representative rearranged T-cell receptor beta gene showing the approximate placement of the upstream and downstream DNA primers. The numbers of primers and their specificity are listed for Master Mix Tubes A, B, and C.

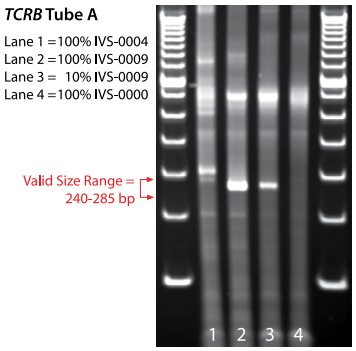
EuroClonality This assay is based on the **EuroClonality/BIOMED-2** Concerted Action BMH4-CT98-3936.

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0009 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0004 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0000 Polyclonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	**Target**	**Units in Assay**	**Units in Assay MegaKit**
TCRB Tube A	Multiple Vβ + Jβ1/2	1 x 1500 µL tube	10 x 1500 µL tubes
TCRB Tube B	Multiple Vβ + Jβ2	1 x 1500 µL tube	10 x 1500 µL tubes
TCRB Tube C	Multiple Dβ + Jβ1/2	1 x 1500 µL tube	10 x 1500 µL tubes
Specimen Control Size Ladder	Multiple Genes	1 x 1500 µL tube	10 x 1500 µL tubes

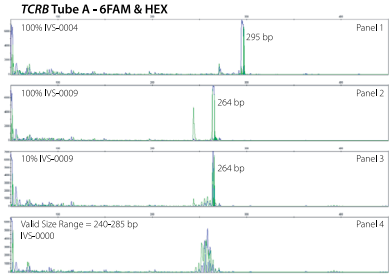
Polyacrylamide Gel Electrophoresis Detection

Heteroduplex analysis is performed to differentiate clonal and non-clonal PCR products. It involves heat denaturation of double-stranded DNA, followed by a snap chilling process to force DNA strands to quickly reanneal. In non-clonal populations, this process causes a majority of the single-stranded DNA to incorrectly bind to non-homologous strands, resulting in secondary structures and reducing the DNA's ability to migrate through a non-denaturing polyacrylamide gel. As a result, polyclonal products are frequently observed as smears at high molecular weights. In clonal populations, after heteroduplex analysis is performed, most denatured single-strand PCR products will reanneal correctly with homologous strands of DNA (reforming homoduplexes), allowing them to easily migrate through the polyacrylamide gel as a single band. Therefore, heteroduplex analysis is key for analyzing PCR products visualized using gel detection methods, as it increases the separation between clonal and polyclonal products.



Capillary Electrophoresis Detection (ABI)

Differential fluorescence detection, such as ABI fluorescence detection, is commonly used to resolve different-sized amplicon products using a capillary electrophoresis instrument. Primers can be conjugated with several different fluorescent dyes (fluorophores), so that they produce different emission spectra upon excitation by a laser in the capillary electrophoresis instrument. In this manner, different fluorescent dyes can correspond to different targeted regions. This detection system results in excellent sensitivity, single nucleotide resolution, differential product detection, and relative quantification. In addition, differential detection allows accurate, reproducible and objective interpretation of primer-specific products. Inter-assay and intra-assay reproducibility in size determination using capillary electrophoresis is approximately 1 to 4 nucleotides.



Ordering Information

Catalog #	Products	Quantity
9-205-0010	IdentiClone™ *TCRB* Clonality Assay - Gel Detection	33 reactions
9-205-0020	IdentiClone™ *TCRB* Clonality Assay MegaKit - Gel Detection	330 reactions
9-205-0011	IdentiClone™ *TCRB* Clonality Assay - ABI Fluorescence Detection	33 reactions
9-205-0021	IdentiClone™ *TCRB* Clonality Assay MegaKit - ABI Fluorescence Detection	330 reactions

These are *in vitro* diagnostic products, and are not available for sale or use within North America.

IdentiClone™

T-Cell Receptor Gamma Gene Rearrangement Assay 2.0

Intended Use

The IdentiClone T-Cell Receptor Gamma Gene Rearrangement Assay (*TCRG* 2.0) is an *in vitro* diagnostic product intended for PCR-based detection of clonal T-cell receptor gamma chain gene rearrangements in patients with suspected lymphoproliferations.

Specifically, the IdentiClone T-Cell Receptor Gamma Gene Rearrangement Assay 2.0 can be used to:

- Identify clonality in suspected lymphoproliferations
- Monitoring and evaluation of disease recurrence
- Evaluation of new research and methods in malignancy studies

Summary and Explanation of the Test

Rearrangements of the antigen receptor genes occur during ontogeny in B- and T-lymphocytes. These gene rearrangements generate products that are unique in length and sequence. Polymerase chain reaction (PCR) assays can be used to identify lymphocyte populations derived from a single cell by detecting the unique V-J gene rearrangements present within these antigen receptor loci[1].

The Invivoscribe designed CE-marked IdentiClone Assays represents an improved approach to PCR-based clonality testing of lymphoproliferative disorders, as it can detect the vast majority of T-cell receptor gamma (*TRG*, formerly known as *TCRG*) gene rearrangements with a single multiplex master mix.

This assay allows for amplification of the *TRG* region with fluorescent labeled primers, yielding products that can be grouped under a single Gaussian distribution when separated by size using capillary electrophoresis. In addition, the product size facilitates increased success when testing FFPE samples. The included analysis algorithm aids in the interpretation of data and identification of significant clonal peaks. Presence or absence of molecular clonality can support the differential diagnosis of reactive lesions and certain B- and T-cell malignancies, provided that the results are interpreted in the context of all available clinical, histological, and immunophenotypic data.

Performance Characteristics

To assess the performance of the *TCRG* 2.0 Assay, testing was performed on cell lines with known clonal rearrangements followed by testing on previously sequenced clinical samples.

When used in combination with the provided *TCRG* Algorithm worksheet, the assay was capable of detecting DNA from 6 control cell lines (200 ng/µL) diluted into polyclonal tonsil DNA (200 ng/µL) at 5% (v/v).

Furthermore, the performance of the *TCRG* 2.0 Assay was evaluated on clinical samples for which the T-cell receptor gamma gene rearrangement status had been identified by Roche 454 sequencing. For the 7 samples that had been identified as clonal by sequencing, the *TCRG* 2.0 assay had 100% concordance. For the 12 samples that were either negative for a clonal event or were oligoclonal, concordance of the *TCRG* 2.0 assay was 75%. Sample types included peripheral blood, bone marrow, and formalin-fixed, paraffin embedded (FFPE) tissue.

The results of molecular clonality tests should always be interpreted in the context of clinical, histological and immunophenotypic data.

Reference

1. Miller JE, Wilson SS, Jaye DJ, and Kronenberg M. *Mol. Diag*. 1999, 4(2):101-117.

EuroClonality

This assay was developed by Invivoscribe.
The performance of this assay was reviewed and validated by the EuroClonality/BIOMED-2 Group.
Euroclonality manuscript in preparation: multicenter study with 250 clinical patient specimens.

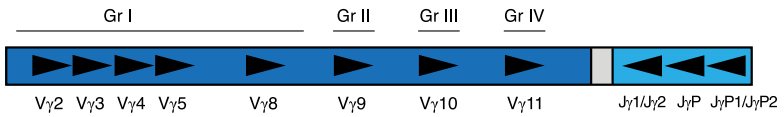


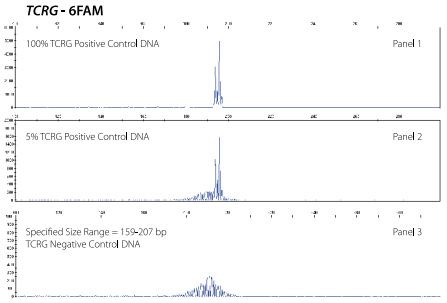
Figure Legend: Simple representation of the organization of the T-cell receptor gamma gene on chromosome 7p14. Black arrows represent the relative positions of primers that target the variable region genes and the downstream joining region gene segments that are involved in rearrangements in T-cell lymphomas. The downstream primers are fluorescently labeled through the incorporation of a 6FAM fluorophore. The amplicon products generated from these rearrangements are detected by capillary electrophoresis.

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
5% *TCRG* Positive Control DNA	50 µg/mL	1 x 50 µL tube	5 x 50 µL tube
TCRG Negative Control DNA	50 µg/mL	1 x 50 µL tube	5 x 50 µL tube
Master Mixes	**Target**	**Units in Assay**	**Units in Assay MegaKit**
TCRG - 6FAM	Vγ1-Vγ11 + Jγ1/Jγ2, JγP, JγP1/JγP2	1 x 1500 µL tube	10 x 1500 µL tubes
Specimen Control Size Ladder	Multiple Genes	1 x 1500 µL tube	10 x 1500 µL tubes

Capillary Electrophoresis Detection (ABI)

Fluorescence detection is commonly used to resolve the different sized amplicon products using a capillary electrophoresis instrument. Primers are conjugated with a 6-FAM fluorescent dye (fluorophore), so that they can be detected upon excitation by laser. This detection system results in a high sensitivity, single nucleotide size resolution, and relative quantification. Inter- and intra-assay reproducibility in size determination using capillary electrophoresis is approximately 1 to 4 nucleotides. The data shown were generated using the *TCRG*–6FAM master mix. Amplified products were run on an ABI 3130 instrument.



Ordering Information

Catalog #	Products	Quantity
9-207-0101	IdentiClone™ T-Cell Receptor Gamma Gene Rearrangement Assay 2.0 - ABI Fluorescence Detection	33 reactions
9-207-0111	IdentiClone™ T-Cell Receptor Gamma Gene Rearrangement Assay MegaKit 2.0 - ABI Fluorescence Detection	330 reactions

These are *in vitro* diagnostic products, and are not available for sale or use within North America.

IdentiClone™

TCRG Gene Clonality Assays

Intended Use

The IdentiClone *TCRG* Gene Clonality Assays are *in vitro* diagnostic products intended for PCR-based detection of clonal T-cell receptor gamma chain gene rearrangements in patients with suspected lymphoproliferations.

Specifically, the IdentiClone *TCRG* Gene Clonality Assays can be used to:

- Identify clonality in suspected lymphoproliferations
- Support a differential diagnosis between reactive lesions and T-cell and some immature B-cell malignancies
- Determine lineage involvement in mature lymphoproliferative disorders
- Monitor and evaluate disease recurrence

Summary and Explanation of the Test

The Invivoscribe CE-marked IdentiClone Assays represent a unique approach to PCR-based clonality testing. These standardized assays were carefully optimized testing positive and negative control samples using multiplex master mixes. Assay development was followed by extensive validation including the testing of more than 400 clinical samples using Revised European/American Lymphoma (REAL) Classification. Testing was done at more than thirty prominent independent testing centers throughout Europe in a collaborative study known as the BIOMED-2 Concerted Action. Results from this BIOMED-2 study appear in *Leukemia*, a leading peer-reviewed journal[1].

These test kits include 3 master mixes targeting *TRG* (formerly known as *TCRG*) gene rearrangements. *TCRG* Tube A contains primers that target the Vγ1-8 + Vγ10 genes and Jγ1.1, Jγ1.3, Jγ2.1, and Jγ2.3 genes (also known as JγP1, Jγ1, JγP2, and Jγ2 respectively). *TCRG* Tube B contains primers that target the Vγ9 + Vγ11 genes and Jγ1.1, Jγ1.3, Jγ2.1, and Jγ2.3 genes. The Specimen Control Size Ladder master mix targets multiple genes and generates a series of amplicons of approximately 100, 200, 300, 400, and 600 base pairs to ensure that the quality and quantity of input DNA is adequate to yield a valid result. A single thermocycler program and similar detection methodologies are used with all of our gene clonality assays. This improves consistency and facilitates cross-training.

Performance Characteristics

Data from an independent, peer-reviewed study suggests the diagnostic accuracy of selected IdentiClone tests to be 96%. There were no clear false-positive results generated using the IdentiClone tests, and there was a high level of precision. The clinico-histopathological diagnosis correlates well with PCR results in a higher number of patients when compared with Southern Blot (SB) results, as seen below:

	PCR/SB concordance[1]:
IGH:	93% sensitivity / 92% specificity
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Reference

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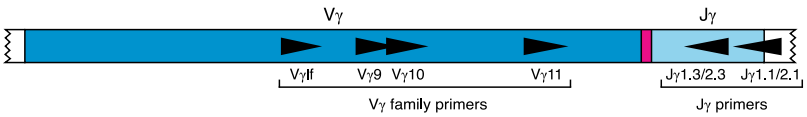


Figure Legend: Simple representation of the organization of the T-cell receptor gamma chain gene on chromosome 7. Black arrows represent the relative positions of primers that target the variable (*Vγ*) regions, and the downstream joining (*Jγ*) gene segments. The amplicon products generated from each of these regions can be differentially detected when fluorescent primer sets are used with capillary electrophoresis instruments that employ differential fluorescence detection.

TCRG tube A: Vγlf and Vγ10 primers + Jγ1.1/2.1 and Jγ1.3/2.3

TCRG tube B: Vγ9 and Vγ11 primers + Jγ1.1/2.1 and Jγ1.3/2.3

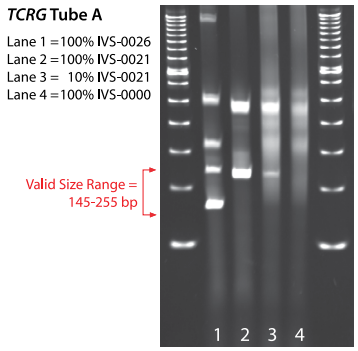
EuroClonality This assay is based on the **EuroClonality/BIOMED-2** Concerted Action BMH4-CT98-3936.

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0009 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0021 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0000 Polyclonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	**Target**	**Units in Assay**	**Units in Assay MegaKit**
TCRG Tube A	Vγ1-8 + Vγ10 + Jγ	1 x 1500 µL tube	10 x 1500 µL tubes
TCRG Tube B	Vγ9 + Vγ11 + Jγ	1 x 1500 µL tube	10 x 1500 µL tubes
Specimen Control Size Ladder	Multiple Genes	1 x 1500 µL tube	10 x 1500 µL tubes

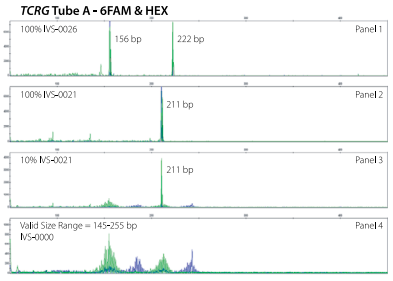
Polyacrylamide Gel Electrophoresis Detection

Heteroduplex analysis is performed to differentiate clonal and non-clonal PCR products. It involves heat denaturation of double-stranded DNA, followed by a snap chilling process to force DNA strands to quickly reanneal. In non-clonal populations, this process causes a majority of the single-stranded DNA to incorrectly bind to non-homologous strands, resulting in secondary structures and reducing the DNA's ability to migrate through a non-denaturing polyacrylamide gel. As a result, polyclonal products are frequently observed as smears at high molecular weights. In clonal populations, after heteroduplex analysis is performed, most denatured single-strand PCR products will reanneal correctly with homologous strands of DNA (reforming homoduplexes), allowing them to easily migrate through the polyacrylamide gel as a single band. Therefore, heteroduplex analysis is key for analyzing PCR products visualized using gel detection methods, as it increases the separation between clonal and polyclonal products.



Capillary Electrophoresis Detection (ABI)

Differential fluorescence detection, such as ABI Fluorescence Detection, is commonly used to resolve different-sized amplicon products using a capillary electrophoresis instrument. Primers can be conjugated with several different fluorescent dyes (fluorophores), so that they produce different emission spectra upon excitation by a laser in the capillary electrophoresis instrument. In this manner, different fluorescent dyes can correspond to different targeted regions. This detection system results in excellent sensitivity, single nucleotide resolution, differential product detection, and relative quantification. In addition, differential detection allows accurate, reproducible and objective interpretation of primer-specific products. Inter-assay and intra-assay reproducibility in size determination using capillary electrophoresis is approximately 1 to 4 nucleotides.



Ordering Information

Catalog #	Products	Quantity
9-207-0020	IdentiClone™ *TCRG* Gene Clonality Assay - Gel Detection	33 reactions
9-207-0040	IdentiClone™ *TCRG* Gene Clonality Assay MegaKit - Gel Detection	330 reactions
9-207-0021	IdentiClone™ *TCRG* Gene Clonality Assay - ABI Fluorescence Detection	33 reactions
9-207-0041	IdentiClone™ *TCRG* Gene Clonality Assay MegaKit - ABI Fluorescence Detection	330 reactions

These are *in vitro* diagnostic products, and are not available for sale or use within North America.

IdentiClone™

TCRD Gene Clonality Assays

Intended Use

The IdentiClone *TCRD* Gene Clonality Assays are *in vitro* diagnostic products intended for PCR-based detection of clonal T-cell receptor delta chain gene rearrangements in patients with suspected lymphoproliferations.

Specifically, the IdentiClone *TCRD* Gene Clonality Assays can be used to:

- Identify clonality in suspected lymphoproliferations
- Support a differential diagnosis between reactive lesions and T-cell and some immature B-cell malignancies
- Determine lineage involvement in mature lymphoproliferative disorders
- Monitor and evaluate disease recurrence

Summary and Explanation of the Test

The Invivoscribe CE-marked IdentiClone Assays represent a unique approach to PCR-based clonality testing. These standardized assays were carefully optimized testing positive and negative control samples using multiplex master mixes. Assay development was followed by extensive validation including the testing of more than 400 clinical samples using Revised European/American Lymphoma (REAL) Classification. Testing was done at more than thirty prominent independent testing centers throughout Europe in a collaborative study known as the BIOMED-2 Concerted Action. Results from this BIOMED-2 study appear in *Leukemia*, a leading peer-reviewed journal[1].

These test kits include 2 master mixes. The *TCRD* tube targets the framework regions within the variable region, the diversity region, and the joining region of the T-cell receptor delta chain locus (*TRD*, formerly known as *TCRD*). The Specimen Control Size Ladder master mix targets multiple genes and generates a series of amplicons of approximately 100, 200, 300, 400, and 600 base pairs to ensure that the quality and quantity of input DNA is adequate to yield a valid result. A single thermocycler program and similar detection methodologies are used with all of our gene clonality assays. This improves consistency and facilitates cross-training.

Figure Legend: Simplified diagram of a representative rearranged T-cell receptor delta gene showing the approximate placement of the upstream and downstream DNA primers. The numbers of primers and their specificity are listed for the *TRD* Tube Master Mix tube.

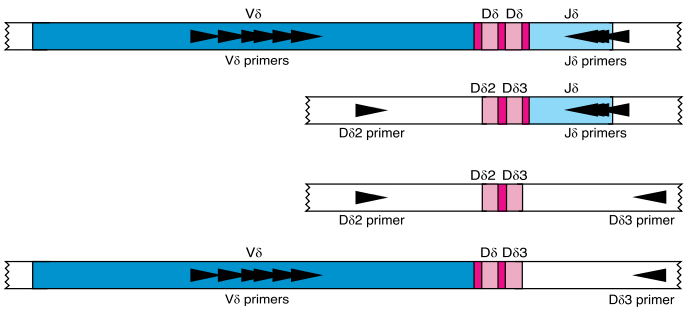
Performance Characteristics

Data from an independent, peer-reviewed study suggests the diagnostic accuracy of selected IdentiClone tests to be 96%. There were no clear false-positive results generated using the IdentiClone tests, and there was a high level of precision. The clinico-histopathological diagnosis correlates well with PCR results in a higher number of patients when compared with Southern Blot (SB) results, as seen below:

	PCR/SB concordance[1]:
IGH:	93% sensitivity / 92% specificity
IGK:	90% sensitivity / 90% specificity
IGL:	86% sensitivity / 92% specificity
TRB:	86% sensitivity / 98% specificity
TRG:	89% sensitivity / 94% specificity
TRD:	83% sensitivity / 95% specificity

Reference

1. JJM van Dongen et al., *Leukemia* 17:2257-2317 (2003).



TCRD tube: 6 Vδ and 1 Dδ2 primers + 4 Jδ and 1 Dδ3 primers

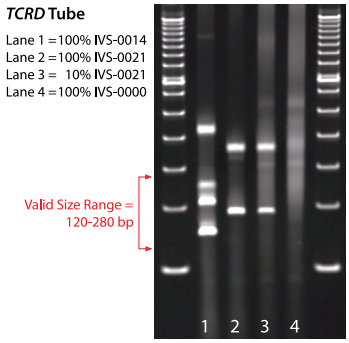
EuroClonality This assay is based on the **EuroClonality/BIOMED-2** Concerted Action BMH4-CT98-3936.

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0021 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0000 Polyclonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	**Target**	**Units in Assay**	**Units in Assay MegaKit**
TCRD Tube	Multiple Vδ + Dδ +Jδ	1 x 1500 µL tube	10 x 1500 µL tubes
Specimen Control Size Ladder	Multiple Genes	1 x 1500 µL tube	10 x 1500 µL tubes

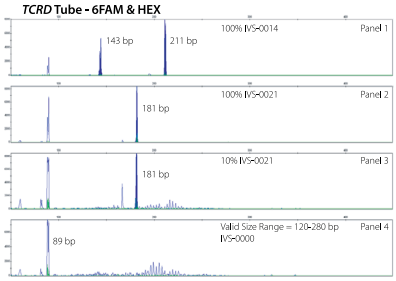
Polyacrylamide Gel Electrophoresis Detection

Heteroduplex analysis is performed to differentiate clonal and non-clonal PCR products. It involves heat denaturation of double-stranded DNA, followed by a snap chilling process to force DNA strands to quickly reanneal. In non-clonal populations, this process causes a majority of the single-stranded DNA to incorrectly bind to non-homologous strands, resulting in secondary structures and reducing the DNA's ability to migrate through a non-denaturing polyacrylamide gel. As a result, polyclonal products are frequently observed as smears at high molecular weights. In clonal populations, after heteroduplex analysis is performed, most denatured single-strand PCR products will reanneal correctly with homologous strands of DNA (reforming homoduplexes), allowing them to easily migrate through the polyacrylamide gel as a single band. Therefore, heteroduplex analysis is key for analyzing PCR products visualized using gel detection methods, as it increases the separation between clonal and polyclonal products.



Capillary Electrophoresis Detection (ABI)

Differential fluorescence detection, such as ABI Fluorescence Detection, is commonly used to resolve different-sized amplicon products using a capillary electrophoresis instrument. Primers can be conjugated with several different fluorescent dyes (fluorophores), so that they produce different emission spectra upon excitation by a laser in the capillary electrophoresis instrument. In this manner, different fluorescent dyes can correspond to different targeted regions. This detection system results in excellent sensitivity, single nucleotide resolution, differential product detection, and relative quantification. In addition, differential detection allows accurate, reproducible and objective interpretation of primer-specific products. Inter-assay and intra-assay reproducibility in size determination using capillary electrophoresis is approximately 1 to 4 nucleotides.



Ordering Information

Catalog #	Products	Quantity
9-206-0010	IdentiClone™ *TCRD* Gene Clonality Assay - Gel Detection	33 reactions
9-206-0020	IdentiClone™ *TCRD* Gene Clonality Assay MegaKit - Gel Detection	330 reactions
9-206-0011	IdentiClone™ *TCRD* Gene Clonality Assay - ABI Fluorescence Detection	33 reactions
9-206-0021	IdentiClone™ *TCRD* Gene Clonality Assay MegaKit - ABI Fluorescence Detection	330 reactions

These are *in vitro* diagnostic products, and are not available for sale or use within North America.

IdentiClone™

*BCL1/*JH Translocation Assay

Intended Use

The IdentiClone *BCL1/*JH Translocation Assay is an *in vitro* diagnostic product intended for PCR-based detection of *IGH-CCND1* (formerly known as *BCL1/*JH) t(11;14)(q13;q32) gene translocations in patients with suspected lymphoproliferations.

Specifically, the IdentiClone *BCL1/*JH Translocation Assay can be used to:

Identify *IGH-CCND1* (formerly known as *BCL1/*JH) gene translocations highly suggestive of mantle cell lymphoma (MCL)

- Distinguish mantle cell lymphoma from other neoplastic or benign B-cell proliferations
- Monitor and evaluate disease recurrence

Summary and Explanation of the Test

The Invivoscribe CE-marked IdentiClone assays represent a unique approach to PCR-based clonality testing. These standardized assays were carefully optimized testing positive and negative control samples using multiplex master mixes. Assay development was followed by extensive validation including the testing of more than 400 clinical samples using Revised European/American Lymphoma (REAL) Classification. Testing was done at more than thirty prominent independent testing centers throughout Europe in a collaborative study known as the BIOMED-2 Concerted Action. Results from this BIOMED-2 study appear in *Leukemia*, a leading peer-reviewed journal[1].

These test kits include includes 2 master mixes. The *BCL1/*JH Tube targets the major translocation cluster (MTC) of the *IGH-CCND1* locus and the joining region of the immunoglobulin heavy chain locus. The Specimen Control Size Ladder master mix targets multiple genes and generates a series of amplicons of approximately 100, 200, 300, 400, and 600 base pairs to ensure that the quality and quantity of input DNA is adequate to yield a valid result. A single thermocycler program and similar detection methodologies are used with many of our assays. This improves consistency and facilitates cross-training.

Performance Characteristics

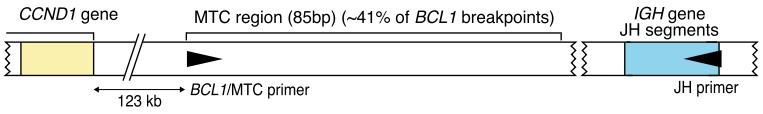
The assay analytical performance was evaluated by testing spiked Mantle Cell Lymphoma (MCL) *IGH-CCND1* positive cell-line DNA into tonsil DNA at six different dilutions. The Limit of Detection (LoD) was observed at 0.1% DNA dilution. To evaluate within-laboratory precision, complete agreement of results was observed across four runs executed by two operators over two days.

Testing conducted across three laboratories using 25 samples from cases of MCL with *IGH-CCND1* translocations and 18 negative samples, showed 100% concordance of positive samples (25 of 25 samples) using fluorescence detection, and 88% (22 of 25 samples) using gel detection. For the negative samples, the concordance was 100% using both gel detection (18 of 18 samples) and fluorescence detection (18 of 18 samples) formats. Specificity for both formats was 100% and sensitivity was determined to be between 10^{-3} and 10^{-4}. The sensitivity is sufficiently high for the detection of the *IGH-CCND1* breakpoint in diagnostic material. However, only 40-50% of the t(11;14) breakpoints in MCL will be detected by PCR alone and additional detection method tools are recommended for diagnosis of breakpoints that do not fall within the major translocation cluster region.

Reference

1. JJM van Dongen et al., *Leukemia* 17:2257-2317 (2003).

Figure Legend: Schematic diagram of the *IGH-CCND1* t(11;14) translocation showing the cyclin D1 (*CCND1*) gene on the left and the Ig heavy chain (*IGH*) gene on the right. Shown are the relative positions and orientations for the *BCL1*/MTC primer and the JH primer, which are included in the *BCL1/*JH Master Mix tube.



t(11;14) tube: 1 *BCL1* MTC primer + 1 JH primer

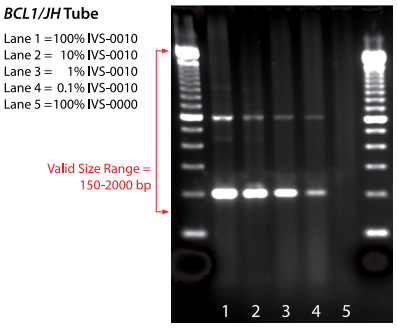
EuroClonality This assay is based on the **EuroClonality/BIOMED-2** Concerted Action BMH4-CT98-3936.

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0010 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0000 Polyclonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	**Target**	**Units in Assay**	**Units in Assay MegaKit**
*BCL1/J*H Tube - Unlabeled	MTC of *BCL1* + *IGH* JH	1 x 1500 µL tube	10 x 1500 µL tubes
Specimen Control Size Ladder - Unlabeled	Multiple Genes	1 x 1500 µL tube	10 x 1500 µL tubes

Polyacrylamide Gel Electrophoresis Detection

Gel electrophoresis, such as agarose gel electrophoresis, is commonly used to resolve different amplicon products based on their size, charge, and conformation. Since DNA is negatively charged, when an electrical potential (voltage) is applied across the gel containing PCR products, the electrical field causes the amplicons to migrate through the gel. Smaller DNA fragments are able to easily migrate through the gel matrix, whereas larger DNA fragments migrate more slowly. This causes a separation of the amplicon products based on size. Ethidium bromide or other DNA intercalating dyes can then be used to stain and detect these products in the gel. The use of a DNA ladder allows the relative size of amplicons to be determined.



Ordering Information

Catalog #	Products	Quantity
9-308-0010	IdentiClone™ *BCL1/J*H Translocation Assay - Gel Detection	33 reactions
9-308-0020	IdentiClone™ *BCL1/J*H Translocation Assay MegaKit - Gel Detection	330 reactions

These are *in vitro* diagnostic products, and are not available for sale or use within North America.

IdentiClone™

BCL2/JH Translocation Assay

Intended Use

The IdentiClone *BCL2*/JH Translocation Assay is an *in vitro* diagnostic product intended for PCR-based detection of *IGH-BCL2* t(14;18) gene translocations in patients with suspected lymphoproliferations.

Specifically, the IdentiClone *BCL2*/JH Translocation Assay can be used to:

- Distinguish lymphoma from benign lymphoid hyperplasia
- Distinguish follicular lymphoma (FL) from other B-cell lymphomas that may have a similar appearance
- Monitor and evaluate disease recurrence

Summary and Explanation of the Test

The Invivoscribe CE-marked IdentiClone Assays represent a unique approach to PCR-based clonality testing. These standardized assays were carefully optimized testing positive and negative control samples using multiplex master mixes. Assay development was followed by extensive validation including the testing of more than 400 clinical samples using Revised European/American Lymphoma (REAL) Classification. Testing was done at more than thirty prominent independent testing centers throughout Europe in a collaborative study known as the BIOMED-2 Concerted Action. Results from this BIOMED-2 study appear in *Leukemia*, a leading peer-reviewed journal[1].

These test kits include 4 master mixes. The *BCL2*/JH Translocation master mixes (*BCL2*/JH Tubes A, B, and C) target the joining (J) region of the immunoglobulin heavy chain (*IGH*) gene and distinct regions of the *BCL2* gene. These master mixes are used to detect major breakpoint region (MBR) and minor cluster region (mcr) of the *IGH-BCL2* t(14;18)(q32;q21) translocations. The Specimen Control Size Ladder master mix targets multiple genes and generates a series of amplicons of approximately 100, 200, 300, 400, and 600 base pairs to ensure that the quality and quantity of input DNA is adequate to yield a valid result. A single thermocycler program and similar detection methodologies are used with many of our assays. This improves consistency and facilitates cross-training.

Performance Characteristics

The initial evaluation of this assay was performed in three laboratories on DNA derived from 124 cases of follicular cell lymphoma (FCL) known to carry the t(14;18) translocation. 109 cases were identified with the *IGH-BCL2* fusion gene (88%) using this PCR assay. The final testing and evaluation was done on samples in 11 independent laboratories[1]. False-positive results (0.4%) were only seen in 12 of 3036 analyses.

This IdentiClone *BCL2*/JH Translocation Assay was found to be more sensitive than Southern blot analysis. Sensitivity differed slightly between the master mixes. However, overall sensitivity for the assay was determined to be between 1 positive cell in 10^2 normal cells and 1 positive cell in 10^3 normal cells.

In conclusion, we have designed and evaluated the performance characteristics of a robust three tube multiplex PCR assay in order to maximize the detection of the t(14;18) breakpoint. This strategy is capable of amplifying across the breakpoint region in the majority of cases of follicular lymphoma with a cytogenetically defined translocation.

Reference

1. JJM van Dongen et al., *Leukemia* 17:2257 - 2317 (2003).

Figure Legend: Schematic diagram of the *IGH-BCL2* t(14;18) translocation showing the *BCL2* gene on the left and the Ig heavy chain (*IGH*) gene on the right. Shown are the relative positions and orientations for the major breakpoint region (MBR) primers, the minor cluster region (mcr) primers, and the JH primer, which are included in the 3 *BCL2*/JH master mix tubes.

t(14;18) tube A: 2 *BCL2* MBR primers + 1 JH primer

t(14;18) tube B: 4 *BCL2* 3'MBR primers + 1 JH primer

t(14;18) tube C: 3 *BCL2* mcr primers + 1 JH primer

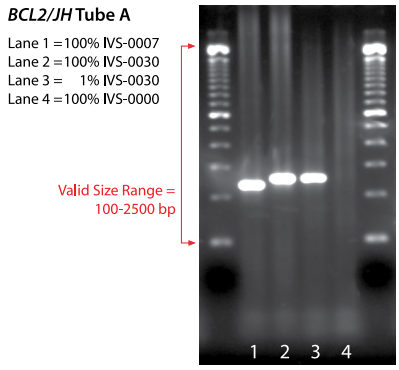
EuroClonality This assay is based on the **EuroClonality/BIOMED-2** Concerted Action BMH4-CT98-3936.

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0030 Clonal Control DNA	100 µL @ 200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-P002 Clonal Control DNA	100 µL @ 1600 pg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0031 Clonal Control DNA	100 µL @ 200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0000 Polyclonal Control DNA	100 µL @ 200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	**Target**	**Units in Assay**	**Units in Assay MegaKit**
BCL2/JH Tube A - Unlabeled	*BCL2* MBR + *IGH* JH	1 x 1500 µL tube	10 x 1500 µL tubes
BCL2/JH Tube B - Unlabeled	*BCL2* 3' MBR + *IGH* JH	1 x 1500 µL tube	10 x 1500 µL tubes
BCL2/JH Tube C - Unlabeled	*BCL2* mcr + *IGH* JH	1 x 1500 µL tube	10 x 1500 µL tubes
Specimen Control Size Ladder - Unlabeled	Multiple Genes	1 x 1500 µL tube	10 x 1500 µL tubes

Polyacrylamide Gel Electrophoresis Detection

Gel electrophoresis, such as agarose gel electrophoresis, is commonly used to resolve different amplicon products based on their size, charge, and conformation. Since DNA is negatively charged, when an electrical potential (voltage) is applied across the gel containing PCR products, the electrical field causes the amplicons to migrate through the gel. Smaller DNA fragments are able to easily migrate through the gel matrix, whereas larger DNA fragments migrate more slowly. This causes a separation of the amplicon products based on size. Ethidium bromide or other DNA intercalating dyes can then be used to stain and detect these products in the gel.



Ordering Information

Catalog #	Products	Quantity
9-309-0020	IdentiClone™ *BCL2*/JH Translocation Assay - Gel Detection	33 reactions
9-309-0040	IdentiClone™ *BCL2*/JH Translocation Assay MegaKit - Gel Detection	330 reactions

These are *in vitro* diagnostic products, and are not available for sale or use within North America.

Warranty and Liability

Invivoscribe Technologies, Inc. (Invivoscribe®) is committed to providing the highest quality products. Invivoscribe® warrants that the products meet or exceed the performance standards described in the Instructions For Use, as to products with such an insert. If a product is covered by product specifications and does not perform as specified, our policy is to replace the product or credit the full purchase price. No other warranties of any kind, expressed or implied, are provided by Invivoscribe®. Invivoscribe® liability shall not exceed the purchase price of the product. Invivoscribe® shall have no liability for direct, indirect, consequential or incidental damages arising from the use, results of use, or inability to use its products; product efficacy under purchaser controlled conditions in purchaser's laboratory must be established and continually monitored through purchaser defined and controlled processes including but not limited to testing of positive, negative, and blank controls every time a sample is tested. Ordering, acceptance, and use of product constitutes purchaser acceptance of sole responsibility for assuring product efficacy and purchaser agreement to the limitation of liability set forth in this paragraph.

NOTICE: The products in the section that follows are *in vitro* diagnostic products and are not available for sale or use within North America. Many of these products are covered by United States patents 6846630 and 8178292, licensed exclusively to Invivoscribe®.

These products require nucleic acid amplification methods such as Polymerase Chain Reaction (PCR). No license under these patents to use amplification processes or enzymes is conveyed expressly or by implication to the purchaser by the purchase of these products.

LeukoStrat® is a registered trademark of Invivoscribe®.

Gel and Capillary

CE IVD Assays

LeukoStrat®

LeukoStrat Assay kits are CE-marked in vitro diagnostic products*.

These assay kits are intended for PCR-based detection of *FLT3* activating mutations in patients with acute myelogenous leukemia (AML) using gel or capillary electrophoresis methods.

These tests include standardized Instructions For Use (IFUs) with interpretation guidelines describing the use of the kit's master mixes and controls. Master mixes are composed of a buffered magnesium chloride solution, deoxynucleotides, and primers targeting the gene segments of interest. These assay master mixes are complete with the exception of Taq DNA polymerase, which is not provided.

These assays are available in regular (33 reactions) and in MegaKit formats (330 reactions), with exception of the LeukoStrat® CDx *FLT3* Mutation Assay, which is only available in regular format.

For more information, please visit www.invivoscribe.com

*NOTICE: LeukoStrat Assays are in vitro diagnostic products and are not available for sale or use within North America. For more information regarding the research use only reagents, please see the Gel & Capillary Research Use Only section.

LeukoStrat®

FLT3 Mutation Assay - Gel Detection

Intended Use

The LeukoStrat® *FLT3* Mutation Assay is an in vitro diagnostic product intended for PCR-based detection of *FLT3* activating mutations in patients with acute myelogenous leukemia (AML).

Specifically, the *FLT3* Mutation Assay can be used to:

- Identify internal tandem duplications (ITD) in the *FLT3* gene
- Identify tyrosine kinase domain (TKD) mutations in the *FLT3* gene

Summary and Explanation of the Test

AML in general has a poor prognosis. Assessment of the mutation status of the *FLT3* (fms related tyrosine kinase 3) receptor gene in karyotype normal AML is the most important prognostic indicator of disease outcome, which is often substantial, as many studies in AML have shown that the presence of *FLT3* activating mutations portends a poor prognosis[1, 2]. For this reason, *FLT3* activation mutation testing is required to stratify disease and determine appropriate treatment options. This LeukoStrat PCR assay targets regions of the *FLT3* gene to identify ITD mutations and TKD mutations, such as the D835 and I836 mutations.

This assay cannot reliably detect *FLT3* mutations comprising less than 5% of the total cell population. It should be emphasized that the results of molecular mutation tests should always be interpreted in the context of clinical, histological and immunophenotypic data.

This test kit includes 3 master mixes. *FLT3* ITD Master mix tests for internal tandem duplication mutations. *FLT3* D835 Master Mix tests for TKD region mutations. The Specimen Control Size Ladder master mix targets multiple genes and generates a series of amplicons of approximately 100, 200, 300, 400, and 600 basepairs to ensure that the quality and quantity of input DNA is adequate to yield a valid result.

Performance Characteristics

This LeukoStrat *FLT3* Mutation Assay offers a rapid and reliable method for detecting *FLT3* mutations. This is shown by a validation performed by the Laboratory for Personalized Molecular Medicine (LabPMM).

	Concordance with three independent labs:
ITD:	100% sensitivity / 100% specificity
TKD:	100% sensitivity / 100% specificity

LabPMM tested 57 blinded patient samples obtained from three independent institutions. The institutions determined that 13 of the samples were *FLT3* ITD positive, 33 were *FLT3* ITD negative, 6 were *FLT3* TKD positive, and 50 were *FLT3* TKD negative. In addition 10 positive blinded spiked samples and 10 negative samples were used for the validation of *FLT3* TKD. The LeukoStrat *FLT3* Mutation Assay showed a sensitivity and specificity of 100% with both master mixes. The analytical sensitivity of both master mixes was determined to be 5 positive cells out of 100 total cells.

Reference

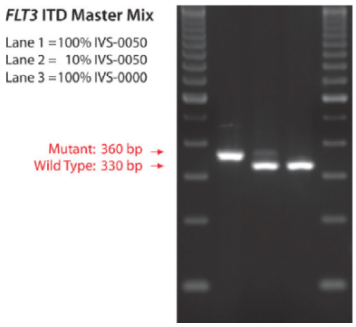
1. Murphy KM et al., A Clinical PCR/Capillary Electrophoresis Assay for the Detection of Internal Tandem Duplication and Point Mutation of the *FLT3* Gene. *J. Mol. Diag.* 5:96-102 (2003).
2. Yamamoto, Y., et al., Activating mutation of D835 within the activation loop of *FLT3* in human hematologic malignancies. *Blood,* 97(8):2434-9 (2001).

Reagents

Controls	Concentration	Units in 33 Reaction Assay	Units in 330 Reaction Assay
IVS-0050 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-P004 Clonal Control DNA	171 pg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0000 Polyclonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	**Target**	**Units in 33 Reaction Assay**	**Units in 330 Assay MegaKit**
FLT3 ITD Master Mix - Unlabeled	*FLT3* ITD	1 x 1500 µL tube	10 x 1500 µL tubes
FLT3 ITD Master Mix - Unlabeled	*FLT3* TKD	1 x 1500 µL tube	10 x 1500 µL tubes
Specimen Control Size Ladder	Multiple Genes	1 x 1500 µL tube	10 x 1500 µL tubes

Polyacrylamide Gel Electrophoresis detection

Gel electrophoresis, such as agarose gel electrophoresis or non-denaturing polyacrylamide gel electrophoresis (PAGE), is commonly used to resolve the different amplicon products based on their size, charge, and conformation. Since DNA is negatively charged, when an electrical potential (voltage) is applied across the gel containing PCR products, the electrical field causes the amplicons to migrate through the gel. Smaller DNA fragments are able to easily migrate through the gel matrix, whereas larger DNA fragments migrate more slowly. This causes a separation of the amplicon products based on size. Ethidium bromide or other DNA intercalating dyes can then be used to stain and detect these products in the gel.



Ordering Information

Catalog #	Products	Quantity
9-412-0010	LeukoStrat™ *FLT3* Mutation Assay - Gel Detection	33 reactions
9-412-0020	LeukoStrat™ *FLT3* Mutation Assay MegaKit - Gel Detection	330 reactions

These are *in vitro* diagnostic products, and are not available for sale or use within North America.

LeukoStrat®

FLT3 Mutation Assay 2.0 - ABI Fluorescence Detection

Intended Use

The LeukoStrat® *FLT3* Mutation Assay 2.0 is an in vitro diagnostic product intended for PCR-based detection of *FLT3* activating mutations in patients with acute myelogenous leukemia (AML).

Specifically, the *FLT3* Mutation Assay can be used to:

- Identify internal tandem duplications (ITD) in the *FLT3* gene
- Identify tyrosine kinase domain (TKD) mutations in the *FLT3* gene

Summary and Explanation of the Test

AML in general has a poor prognosis[1,2]. Assessment of the mutation status of the *FLT3* (fms related tyrosine kinase 3) receptor gene is the most important prognostic indicator of disease outcome, occurring in approximately 30% of patients at the time of diagnosis[3]. For this reason, testing for *FLT3* activating mutations is required for the stratification of disease and determination of appropriate treatment options. The LeukoStrat *FLT3* Mutation Assay 2.0 is a PCR-based method that identifies ITD and TKD mutations. This test kit includes 2 master mixes: the *FLT3*-ITD Master Mix for the detection of ITD mutations and *FLT3*-D835 Master Mix for the detection of TKD region mutations (such as the D835 and I836 mutations).

Performance Characteristics

The LeukoStrat *FLT3* Mutation Assay 2.0 is a rapid and reliable method for the detection of *FLT3* mutations, as evidenced by comparison with Roche® 454 sequencing.

The *FLT3* Mutation Assay 2.0 is capable of detecting *FLT3*-ITD and TKD mutations with excellent concordance (Table 1 and 2) and has high reliability when multiple standard laboratory variables are considered, including multiple operators, reagent lots, different ABI 3500xL instruments, and nonconsecutive testing days.

Table1. *FLT3* ITD Percent Agreement with 454 Sequencing

Percent Agreement		Discordance #	Concordance #	*95% LL
Negative PA	100%	0	119	96.9%
Positive PA	98.0%	4	200	95.1%

*95% of results would be expected to agree with sequencing at a rate greater than or equal to the lower limit (LL).

Table2. *FLT3* TKD Percent Agreement with 454 Sequencing

Percent Agreement		Discordance #	Concordance #	*95% LL
Negative PA	100%	0	137	96.9%
Positive PA	100%	0	240	98.5%

*95% of results would be expected to agree with sequencing at a rate greater than or equal to the lower limit (LL).

Reference

1. Murphy KM et al., A Clinical PCR/Capillary Electrophoresis Assay for the Detection of Internal Tandem Duplication and Point Mutation of the *FLT3* Gene. *J. Mol. Diag.* 5:96-102 (2003).
2. Yamamoto, Y., et al., Activating mutation of D835 within the activation loop of *FLT3* in human hematologic malignancies. *Blood*, 97(8):2434-9 (2001).
3. Acute Myeloid Leukemia, Clinical Practice Guidelines in Oncology, National Comprehensive Cancer Network (v.2.2014)

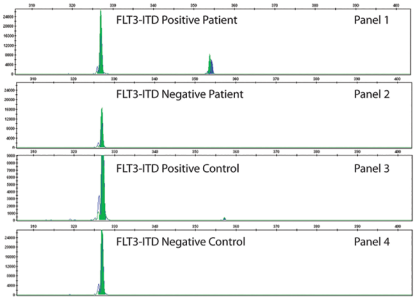
Reagents

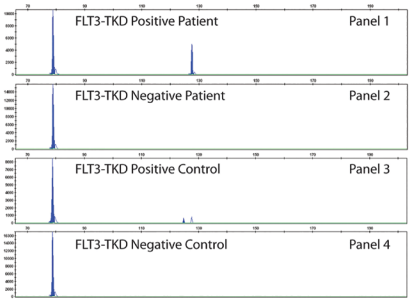
Controls	Concentration	Units in Assay	Units in MegaKit
FLT3 ITD Positive Control	50 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
FLT3 D835 Positive Control	50 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
FLT3 Negative Control	50 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	**Target**	**Units in Assay**	**Units in MegaKit**
FLT3 ITD Master Mix – 6FAM & HEX	*FLT3* ITD	1 x 1500 µL tube	10 x 1500 µL tubes
FLT3 D835 Master Mix – 6FAM	*FLT3* TKD	1 x 1500 µL tube	10 x 1500 µL tubes

Capillary Electrophoresis Detection (ABI)

Differential fluorescence detection, such as ABI fluorescence detection, is commonly used to resolve different-sized amplicon products using a capillary electrophoresis instrument. Primers can be conjugated with different fluorescent dyes (fluorophores), so that they produce different emission spectra upon excitation by a laser in the capillary electrophoresis instrument. In this manner, different fluorescent dyes can correspond to different targeted regions. This detection system results in high sensitivity, single nucleotide resolution, differential product detection, and relative quantification. In addition, differential detection allows accurate, reproducible and objective interpretation of primer-specific products. Inter-assay and intra-assay reproducibility in size determination using capillary electrophoresis is approximately 1 to 4 nucleotides.

The data shown was generated using the master mixes indicated. Amplified products were run on an ABI 3500xL instrument.





Ordering Information

Catalog #	Products	Quantity
9-412-0091	LeukoStrat® *FLT3* Mutation Assay 2.0 – ABI Fluorescence Detection	33 reactions
9-412-0101	LeukoStrat® *FLT3* Mutation Assay 2.0 MegaKit – ABI Fluorescence Detection	330 reactions

These are *in vitro* diagnostic products, and are not available for sale or use within North America.

LeukoStrat®

NEW

CDx *FLT3* Mutation Assay

The only internationally standardized CE-IVD assay for *FLT3* Signal Ratio mutation analysis for selection of acute myeloid leukemia (AML) patients eligible for treatment with midostaurin.

Intended Use

The LeukoStrat® CDx *FLT3* Mutation Assay is a PCR-based in vitro diagnostic test designed to detect internal tandem duplications (ITD) *FLT3* mutations and tyrosine kinase domain (TKD) *FLT3* mutations in genomic DNA extracted from mononuclear cells obtained from peripheral blood or bone marrow aspirates of patients diagnosed with acute myelogenous leukemia.

The LeukoStrat CDx *FLT3* Mutation Assay is used as an aid in the assessment of patients for whom RYDAPT® (midostaurin) treatment is being considered.

Summary and Explanation of the Test

AML in general has a poor prognosis. Assessment of the mutation status of the *FLT3* (fms related tyrosine kinase 3) receptor gene in karyotype normal AML is the most important prognostic indicator of disease outcome, which is often substantial, as many studies in AML have shown that the presence of *FLT3* activating mutations portends a poor prognosis[1,2]. The LeukoStrat CDx *FLT3* Mutation Assay targets regions of the *FLT3* gene to identify ITD mutations and TKD mutations, such as the D835 and I836 mutations, and has been validated in an international clinical trial.

The LeukoStrat CDx *FLT3* Mutation Assay includes reagents, equipment, software and procedures for isolating mononuclear cells and extracting DNA from patient specimens to determine if *FLT3* mutations are present. DNA is amplified via PCR and the amplicons are detected via capillary electrophoresis. *FLT3* mutation status is determined by the LeukoStrat CDx *FLT3* Software. A *FLT3* ITD and/or TKD mutation is reported as Positive if the mutant:wild-type signal ratio meets or exceeds the clinical cutoff of 0.05.

Method Description

ITD Mutations of *FLT3*

The LeukoStrat CDx *FLT3* Mutation Assay uses fluorescently labeled primers that are in the JM region. Wild-type *FLT3* alleles will amplify and produce a product at 327±1 bp as measured by this assay, while alleles that contain ITD mutations will produce a product that exceeds 330±1 bp (Figure 1).

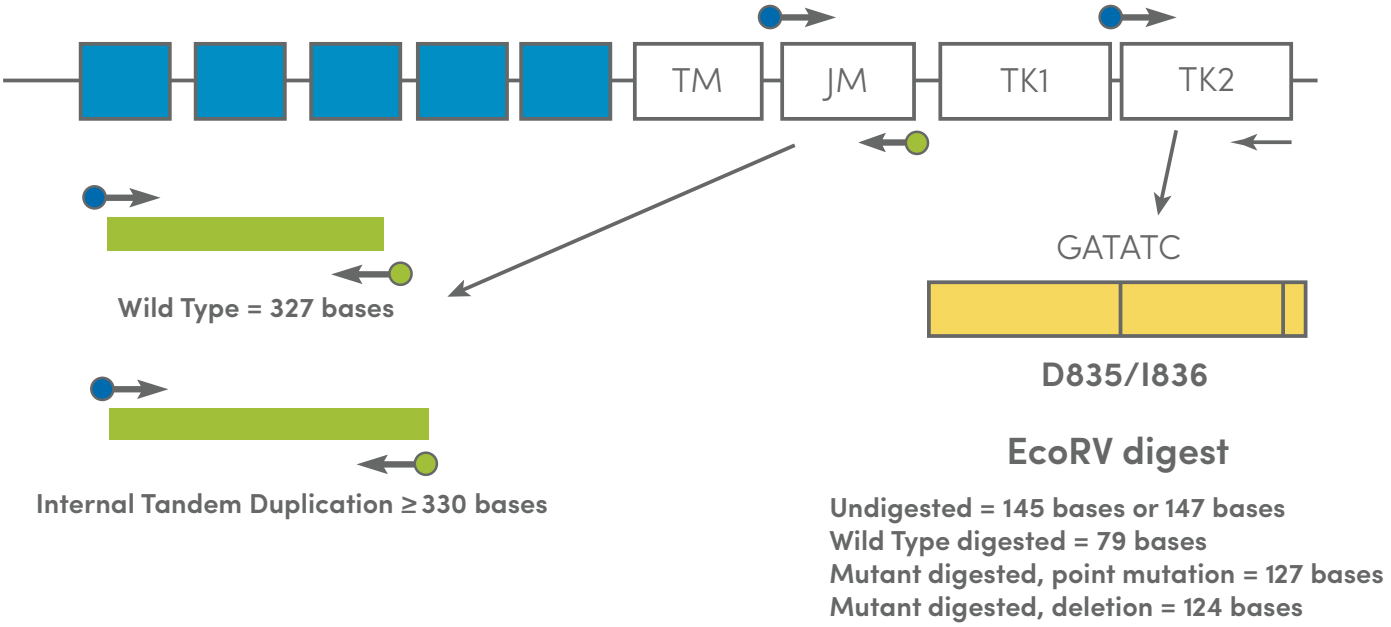
TKD Mutations of *FLT3*

The LeukoStrat CDx *FLT3* Mutation Assay uses primers that lie on either side of the TKD region. The *FLT3* target region is amplified using PCR and then an EcoRV restriction digest is performed. Wild-type alleles of the *FLT3* gene yield digestion products of 79±1 bp whereas mutant alleles yield products of 125±1 bp or 127±1 bp from the original undigested amplicon product of 145±1 bp or 147±1 bp, as measured by this assay (Figure 1).

Reference

1. Murphy KM et al., A Clinical PCR/Capillary Electrophoresis Assay for the Detection of Internal Tandem Duplication and Point Mutation of the *FLT3* Gene. *J. Mol. Diag.* 5:96-102 (2003).
2. Yamamoto, Y., et al., Activating mutation of D835 within the activation loop of *FLT3* in human hematologic malignancies. *Blood*, 97(8):2434-9 (2001).

Figure 1



Depicted is a representation of the *FLT3* juxtamembrane (JM) region (TM = transmembrane) and the activating loop of the tyrosine kinase (TK) domain. Black arrows represent the relative positions of primers that target the JM region for ITD or the activating loop of the kinase domain for TKD. Colored dots represent fluorophores on labeled primers. The yellow box has vertical black lines that represent the position of the EcoRV restriction digest sites.

Reagents

Reagent Name	Units in Assay
FLT3 Extraction Control	1 x 1800 µL tube
FLT3 ITD Master Mix	1 x 1500 µL tube
FLT3 TKD Master Mix	1 x 1500 µL tube
FLT3 ITD Positive Control	1 x 100 µL tube
FLT3 TKD Positive Control	1 x 100 µL tube
FLT3 No Template Control	1 x 200 µL tube

All reagents should be stored at -15 to -30 degrees C.

Ordering Information

Catalog #	Products	Quantity
K-412-0291	LeukoStrat® CDx *FLT3* Mutation Assay	33 reactions
K-412-0281	LeukoStrat® CDx *FLT3* Mutation Assay Software	1 CD complimentary with purchase

These are *in vitro* diagnostic products, and are not available for sale or use within North America.

Warranty and Liability

Invivoscribe Technologies, Inc. (Invivoscribe®) is committed to providing the highest quality products. Invivoscribe® warrants that the products meet or exceed the performance standards described in the Instructions For Use, as to products with such an insert. If a product is covered by product specifications and does not perform as specified, our policy is to replace the product or credit the full purchase price. No other warranties of any kind, expressed or implied, are provided by Invivoscribe®. Invivoscribe® liability shall not exceed the purchase price of the product. Invivoscribe shall have no liability for direct, indirect, consequential or incidental damages arising from the use, results of use, or inability to use its products; product efficacy under purchaser controlled conditions in purchaser's laboratory must be established and continually monitored through purchaser defined and controlled processes including but not limited to testing of positive, negative, and blank controls every time a sample is tested. Ordering, acceptance, and use of product constitutes purchaser acceptance of sole responsibility for assuring product efficacy and purchaser agreement to the limitation of liability set forth in this paragraph.

NOTICE: Many of these products in the section that follows are covered by one or more of the following: European Patent Number 1549764, European Patent Number 2418287, European Patent Number 2460889, Japanese Patent Number 4708029, United States Patent 8859748, and related pending and future applications. All of these patents and applications are licensed exclusively to Invivoscribe Technologies, Inc. Additional patents licensed to Invivoscribe covering some of these products apply elsewhere.

These products require nucleic acid amplification methods such as Polymerase Chain Reaction (PCR). No license under these patents to use amplification processes or enzymes is conveyed expressly or by implication to the purchaser by the purchase of these products.

Gel and Capillary

Research Use Only (RUO) Assays

Invivoscribe offers an array of assays for B- and T-cell gene clonality/rearrangements, mutations, and chromosome translocations for the assessment of hematologic malignancies. These Research Use Only (RUO) assays are available for either ABI capillary electrophoresis fluorescence, or PAGE/agarose gel detection, and contain the PCR master mixes, recommended controls, and Instructions For Use.

On the following pages, you will find detailed information on each RUO assay, including: assay use, background information, typical output data, kit contents, and ordering information. These assays are available in regular size (30 or 33 reactions) or high-volume MegaKit formats (300 or 330 reactions).

Please note that RUO assays are not for sale in Europe and other global markets, where products have been registered if an equivalent CE-IVD product targeting the same locus is available. Refer to the preceding pages for information regarding our IdentiClone™ and LeukoStrat® CE-IVD Assays.

For more information, please visit www.invivoscribe.com

IGH + *IGK* B-Cell Clonality Assays

Assay Use

IGH + *IGK* B-Cell Clonality Assays are useful for studies involving:

- Identification of clonal B-cell populations highly suggestive of B-cell malignancies
- Lineage determination of leukemias and lymphomas
- Monitoring and evaluation of disease recurrence
- Detection and assessment of residual disease
- Evaluation of new research and methods in malignancy studies

Summary and Explanation of the Test

Five PCR master mixes are included in these test kits to test for rearrangements of both *IGH* and *IGK*. *IGH* Tubes A, B, and C target the conserved framework 1, 2, and 3 regions (respectively) within the variable (VH) region and the joining (JH) region of the *IGH* locus. *IGK* tubes A and B target the variable (Vκ), intragenic and joining (Jκ), and kappa deleting element (Kde) regions of the *IGK* locus.

Positive and negative controls, as well as Specimen Control Size Ladder Master Mix are included. PCR products can be analyzed by capillary electrophoresis or heteroduplex analysis. Clonality is indicated if any one of the master mixes generates clonal products.

Background

The immunoglobulin heavy chain (*IGH*) gene locus on chromosome 14 (14q32.33, formerly 14q32.3) includes 46–52 functional and 30 nonfunctional variable (VH), 27 functional diversity (DH), and 6 functional joining (JH) gene segments spread over 1250 kilobases[1,2]. The most frequently used VH gene segments in normal and malignant B cells belong to VH3, VH4, and VH1 families, which together cover 75–95% of VH usage. The VH gene segments contain three framework regions (FR) and two complementarity determining regions (CDR). The FRs are characterized by their similarity among the various VH segments, whereas the CDRs are highly different even within the same VH family. The CDRs represent the preferred target sequences for somatic hypermutations; however, somatic mutations can also occur in the FRs. Therefore, family-specific primers in the three different FRs were designed to increase the detection rate of clonal *IGH* B-cell populations and decrease the occurrence of false-negative results due to somatic hypermutation in primer binding sites[1].

The human immunoglobulin kappa (*IGK*) light chain locus on the short arm of chromosome 2 (2p11.2) spans 1820 kb. It is made up of 76 variable (Vκ) gene segments belonging to 7 subgroups, 5 joining (Jκ) gene segments, and one constant (Cκ) gene segment. Productive assembly of the kappa gene is successful in about 60% of human B lymphocytes[2]; however, even when unsuccessful, clonal B cells generally retain the rearranged kappa genes. The Vκ segments encode the first 95 N-terminal amino acids. Positions 96–108 are encoded by one of five joining (Jκ) gene segments. The constant (Cκ) portion of the kappa light chain (amino acids 109–214) is encoded by a single constant (Cκ) region separated from the Jκ region by an intron.

The length of the hypervariable CDR3 in kappa light chain genes is limited and rearrangements in this region display significant skewing (platykurtosis)[3]. Therefore, clonal CDR3 products generated from this region are easily and reliably identified by heteroduplex analysis or capillary electrophoresis.

The performance characteristics of this assay have been independently determined by the EuroClonality/BIOMED-2 Group. This consortium of 47 molecular diagnostic laboratories tested hundreds of clinical samples, taking into account their lymphoproliferative status as defined by the WHO classifications[4].

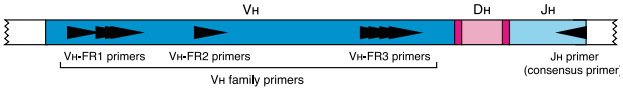
Specimen Requirements

1. 5 mL of peripheral blood, bone marrow biopsy, or bone marrow aspirate anti-coagulated with heparin or EDTA; or,
2. Minimum 5 mm cube of tissue; or,
3. 2 µg of genomic DNA; or,
4. Formalin-fixed, paraffin-embedded (FFPE) tissue or slides.

Reference

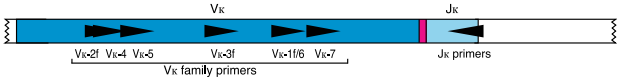
1. M Hummel et al., *Leukemia* 17: 2266–2272 (2003).
2. AW Langerak et al., *Leukemia* 17: 2272–2275 (2003).
3. EP Rock, PR Sibbald, MM Davis, and YH Chien. *J. Exp. Med.* 179(1): 323–328 (1994).
4. JJM van Dongen et al., *Leukemia* 17: 2257–2317 (2003).

EuroClonality **This assay is based on the EuroClonality/BIOMED-2 Concerted Action BMH4-CT98-3936.**

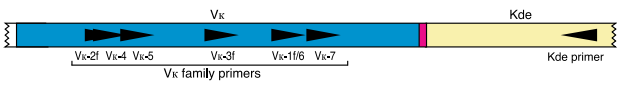


IGH Tube A: 6 VH-FR1 Primers + JH Consensus Primer
IGH Tube B: 7 VH-FR2 Primers + JH Consensus Primer
IGH Tube C: 7 VH-FR3 Primers + JH Consensus Primer

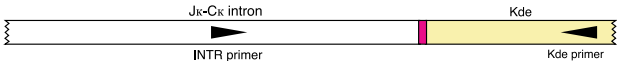
Figure Legend: Simple representation of the organization of a rearranged immunoglobulin heavy chain (*IGH*) gene on chromosome 14 and the immunoglobulin kappa light chain gene on chromosome 2p11.2. Black arrows represent the relative positions of primers that target the conserved framework regions (FR1–3) and the downstream consensus JH gene segments for *IGH* and the Vκ, Jκ, INTR and Kde primers which are included in the *IGK* master mix tubes.



IGK tube A: 6 Vκ primers + 2 Jκ primers



IGK tube B: 6 Vκ primers and INTR primer + 1 κde primer

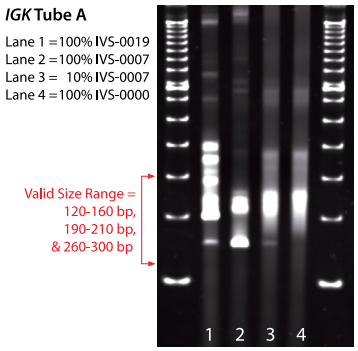


Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0030 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0019 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0007 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0000 Polyclonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	**Target**	**Units in Assay**	**Units in Assay MegaKit**
IGH Tube A	Framework 1 + JH	1 x 1500 µL tube	10 x 1500 µL tubes
IGH Tube B	Framework 2 + JH	1 x 1500 µL tube	10 x 1500 µL tubes
IGH Tube C	Framework 3 + JH	1 x 1500 µL tube	10 x 1500 µL tubes
IGK Tube A	Vk-Jk	1 x 1500 µL tube	10 x 1500 µL tubes
IGK Tube B	Vk-Kde	1 x 1500 µL tube	10 x 1500 µL tubes
Specimen Control Size Ladder	Multiple Genes	1 x 1500 µL tube	10 x 1500 µL tubes

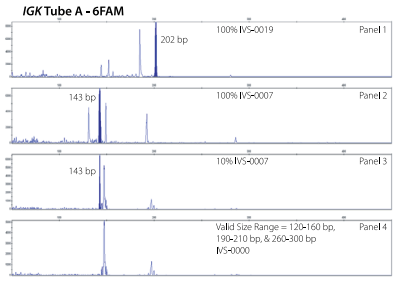
Polyacrylamide Gel Electrophoresis Detection

The data shown was generated using the master mix indicated. Amplified products were heteroduplexed and then run on a 6% polyacrylamide/TBE gel. Lane 1 is data generated testing an alternative 100% clonal control DNA; lane 2 is generated testing the recommended 100% clonal control DNA; lane 3 is data generated testing a 10% dilution of the recommended clonal control DNA; and, lane 4 is data generated testing IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). This DNA is used as a negative control for many of our tests. A standard 100 base pair DNA size ladder was run in the lanes flanking the test samples.



Capillary Electrophoresis Detection (ABI)

The data shown was generated using the master mix indicated. Amplified products were run on an ABI 3100 instrument. For the master mix: Panel 1 displays data generated testing an alternative 100% clonal control DNA; panel 2 displays data generated testing the recommended 100% clonal control DNA; panel 3 displays data generated testing a 10% dilution of the recommended clonal control DNA; and, panel 4 displays data generated testing the IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). Our IVS-0000 DNA is often tested to provide information regarding valid size ranges for each master mix.



Ordering Information

Catalog #	Products	Quantity
1-100-0010	*IGH* + *IGK* B-Cell Clonality Assay - Gel Detection	33 reactions
1-100-0020	*IGH* + *IGK* B-Cell Clonality Assay MegaKit - Gel Detection	330 reactions
1-100-0031	*IGH* + *IGK* B-Cell Clonality Assay - ABI Fluorescence Detection	33 reactions
1-100-0041	*IGH* + *IGK* B-Cell Clonality Assay MegaKit - ABI Fluorescence Detection	330 reactions

IGH Gene Rearrangement Assays

Assay Use

IGH Gene Rearrangement Assays are useful for studies involving:

- Identification of clonal B-cell populations highly suggestive of B-cell malignancies
- Lineage determination of leukemias and lymphomas
- Monitoring and evaluation of disease recurrence
- Detection and assessment of residual disease
- Evaluation of new research and methods in malignancy studies

Summary and Explanation of the Test

Genomic DNA is amplified using three PCR master mixes that target the three conserved framework regions (FR1, FR2, and FR3) of the *IGH* gene and the joining (J_H) region. These regions flank the unique, hypervariable, antigen-binding, complementarity determining region 3 (CDR3). All positive and negative DNA controls, as well as an Amplification Control master mix, are included. The limit of detection of this assay is one clonal B cell in a background of a hundred normal cells. PCR products can be analyzed by capillary electrophoresis or standard gel electrophoresis with ethidium bromide staining. Clonality is indicated if one or more of the three framework master mixes generates clonal products.

Background

Genes encoding immunoglobulin heavy chain (IGH) molecules are assembled from multiple polymorphic gene segments that undergo rearrangement and selection during B-cell development[2]. Rearrangement of these variable (V_H), diversity (D_H), and joining (J_H) genetic segments result in VDJ products of unique length and sequence[1,2]. Clonal *IGH* rearrangements can be rapidly identified through analyses of the size distributions of DNA products amplified from conserved sequences that flank this region[1]. For example, DNA isolated from a normal polyclonal population of B cells produces a Gaussian distribution (bell-shaped size curve) of amplified products; whereas, DNA amplified from a clonal B-cell population generates one or two product(s) of unique size that reflect proliferation of a single rearranged clone[1]. In comparison, southern blot analysis requires 1-2 weeks, is significantly less sensitive, and requires approximately one hundred times more DNA than PCR-based assays, which can be completed in 4-5 hours[1]. In addition, tests of samples previously designated Quantity Not Sufficient (QNS), such as formalin-fixed, paraffin embedded (FFPE) tissue sections, routinely produce a valid result with PCR methods.

Specimen Requirements

1. 5 mL of peripheral blood, bone marrow biopsy, or bone marrow aspirate anti-coagulated with heparin or EDTA; or,
2. Minimum 5 mm cube of tissue; or,
3. 2 µg of genomic DNA; or,
4. Formalin-fixed, paraffin-embedded tissue or slides.

Reference

1. JE Miller, SS Wilson, DL Jaye, and M Kronenberg. *J. Mol. Diag.* 4: 101-117 (1999).
2. S Tonegawa. *Nature* 302: 575-581 (1983).

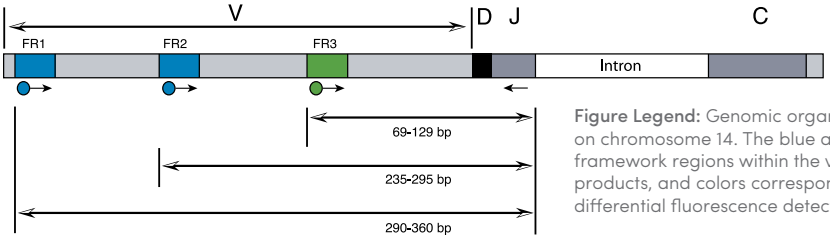


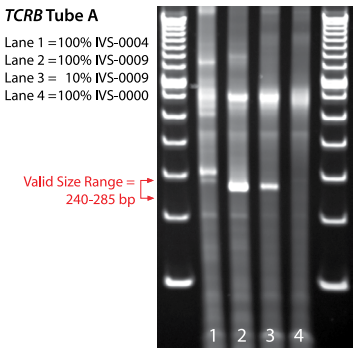
Figure Legend: Genomic organization of a rearranged immunoglobulin heavy chain gene on chromosome 14. The blue and green arrows represent primers targeting the conserved framework regions within the variable region gene. The relative location, size range of valid products, and colors correspond to the products generated from each of these regions when differential fluorescence detection methods are used.

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0030 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0029 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0000 Polyclonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	**Target**	**Units in Assay**	**Units in Assay MegaKit**
IGH Framework 1	Framework 1 + JH	1 x 1500 µL tube	10 x 1500 µL tubes
IGH Framework 2	Framework 2 + JH	1 x 1500 µL tube	10 x 1500 µL tubes
IGH Framework 3	Framework 3 + JH	1 x 1500 µL tube	10 x 1500 µL tubes
Amplification Control	*HLA-DQa*	1 x 1500 µL tube	10 x 1500 µL tubes

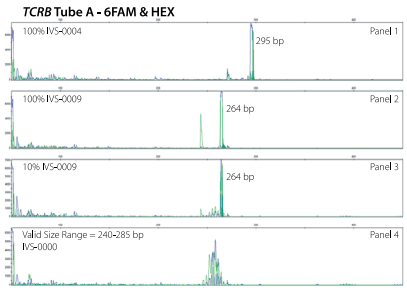
Polyacrylamide Gel Electrophoresis Detection

The data shown was generated using the master mix indicated. Amplified products were run on a 6% polyacrylamide/TBE gel. Lane 1 is data generated testing an alternative 100% clonal control DNA; lane 2 is data generated testing the recommended 100% clonal control DNA; lane 3 is data generated testing a 10% dilution of the recommended clonal control DNA; and, lane 4 is data generated testing IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). IVS-0000 is used as a negative control for many of our tests. A standard 100 base pair DNA size ladder was run in the lanes flanking the test samples.



Capillary Electrophoresis Detection (ABI)

The data shown was generated using the master mix indicated. Amplified products were run on an ABI 3100 instrument. Panel 1 displays data generated testing an alternative 100% clonal control DNA; panel 2 displays data generated testing the recommended 100% clonal control DNA; panel 3 displays data generated testing a 10% dilution of the recommended clonal control DNA; and, panel 4 displays data generated testing the IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). Our IVS-0000 DNA is often tested to provide information regarding valid size ranges for each master mix.



Ordering Information

Catalog #	Products	Quantity
1-101-0010	*IGH* Gene Rearrangement Assay - Gel Detection	33 reactions
1-101-0030	*IGH* Gene Rearrangement Assay MegaKit - Gel Detection	330 reactions
1-101-0051	*IGH* Gene Rearrangement Assay - ABI Fluorescence Detection	33 reactions
1-101-0071	*IGH* Gene Rearrangement Assay MegaKit - ABI Fluorescence Detection	330 reactions

IGH Gene Clonality Assays

Assay Use

IGH Gene Clonality Assays are useful for studies involving:

- Identification of clonal B-cell populations highly suggestive of B-cell malignancies
- Lineage determination of leukemias and lymphomas
- Monitoring and evaluation of disease recurrence
- Detection and assessment of residual disease
- Evaluation of new research and methods in malignancy studies

Summary and Explanation of the Test

Five master mixes target conserved regions within the variable (VH), diversity (DH), and the joining (JH) regions that flank the unique hypervariable, antigen-binding, complementarity determining region 3 (CDR3). Tube A contains six framework region 1 (FR1) primers and a consensus JH region primer. Tube B contains seven framework region 2 (FR2) primers and a consensus JH primer. Tube C contains seven framework region 3 (FR1) primers and a consensus JH primer. Tube D contains six DH region primers and a consensus JH region primer. Tube E contains a DH7 region primer and a consensus JH primer. Positive and negative controls, as well as the Specimen Control Size Ladder Master Mix are included. PCR products can be analyzed by capillary electrophoresis or heteroduplex analysis. Clonality is indicated if any one of the master mixes generates a clonal product.

Background

The immunoglobulin heavy chain (*IGH*) gene locus on chromosome 14 (14q32.33, formerly 14q32.3) includes 46–52 functional and 30 nonfunctional variable (VH), 27 functional diversity (DH), and 6 functional joining (JH) gene segments spread over 1250 kilobases[1,2]. The most frequently used VH gene segments in normal and malignant B cells belong to the VH3, VH4, and VH1 family, together covering 75–95% of VH usage. The VH gene segments contain three framework regions (FR) and two complementarity determining regions (CDR).

The FRs are characterized by their similarity among the various VH segments, whereas the CDRs are highly different even within the same VH family. The CDRs represent the preferred target sequences for somatic hypermutations; however, somatic mutations can also occur in the FRs. Therefore, family-specific primers in the three different FRs were designed to increase the detection rate of clonal *IGH* B-cell populations and decrease the occurrence of false-negative results due to somatic hypermutation in primer binding sites[1]. In addition to VH-JH rearrangements, incomplete DH-JH rearrangements have been found in mature and immature B-cell malignancies. Therefore, DH-JH PCR analysis may be of added value for clonality assessment[2]. The performance characteristics of this assay have been independently determined by the EuroClonality/BIOMED-2 Group. This consortium of 47 molecular diagnostic laboratories tested hundreds of clinical samples, taking into account their lymphoproliferative status as defined by the WHO classifications[3].

Specimen Requirements

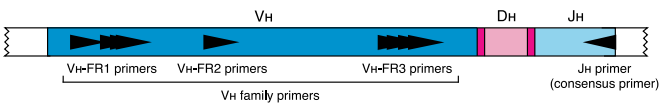
1. 5 mL of peripheral blood, bone marrow biopsy, or bone marrow aspirate anti-coagulated with heparin or EDTA; or,
2. Minimum 5 mm cube of tissue; or,
3. 2 µg of genomic DNA; or,
4. Formalin-fixed, paraffin-embedded (FFPE) tissue or slides.

Reference

1. M Hummel et al., *Leukemia* 17:2266-2272 (2003).
2. AW Langerak et al., *Leukemia* 17:2272-2275 (2003).
3. JJM van Dongen et al., *Leukemia* 17:2257-2317 (2003).

EuroClonality **This assay is based on the EuroClonality/BIOMED-2 Concerted Action BMH4-CT98-3936.**

Figure Legend: Simple representation of the organization of a rearranged immunoglobulin heavy chain gene on chromosome 14. Black arrows represent the relative positions of primers that target the conserved framework (FR1-3) and diversity (DH1-7) regions, and the downstream consensus JH gene segments. The amplicon products generated from each of these regions can be differentially detected when fluorescent primer sets are used with capillary electrophoresis instruments that employ differential fluorescence detection.



Tube A: 6 V_H -FR1 Primers + J_H Consensus Primer
Tube B: 7 V_H -FR2 Primers + J_H Consensus Primer
Tube C: 7 V_H -FR3 Primers + J_H Consensus Primer

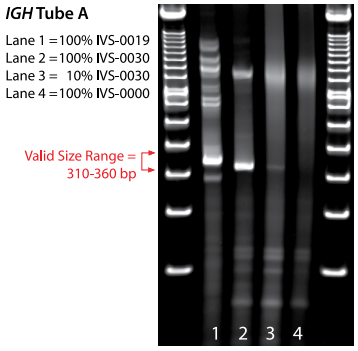
Tube D: 6 D_H Primers + J_H Consensus Primer
Tube E: D_H 7 Primer + J_H Consensus Primer

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0030 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0019 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0024 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0008 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0000 Polyclonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	**Target**	**Units in Assay**	**Units in Assay MegaKit**
IGH Tube A	Framework 1 + JH	1 x 1500 µL tube	10 x 1500 µL tubes
IGH Tube B	Framework 2 + JH	1 x 1500 µL tube	10 x 1500 µL tubes
IGH Tube C	Framework 3 + JH	1 x 1500 µL tube	10 x 1500 µL tubes
IGH Tube D	DH1-6 + JH	1 x 1500 µL tube	10 x 1500 µL tubes
IGH Tube E	DH7 + JH	1 x 1500 µL tube	10 x 1500 µL tubes
Specimen Control Size Ladder	Multiple Genes	1 x 1500 µL tube	10 x 1500 µL tubes

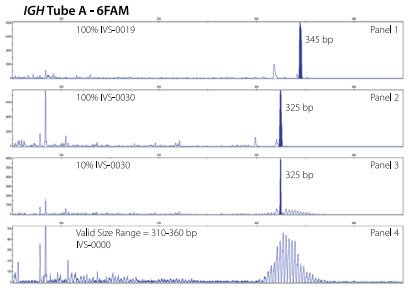
Polyacrylamide Gel Electrophoresis Detection

The data shown was generated using the master mix indicated. Amplified products were heteroduplexed and then run on a 6% polyacrylamide/TBE gel. Lane 1 is data generated testing an alternative 100% clonal control DNA; lane 2 data is generated testing the recommended 100% clonal control DNA; lane 3 is data generated testing a 10% dilution of the recommended clonal control DNA; and, lane 4 is data generated testing IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). IVS-0000 is used as a negative control for many of our tests. A standard 100 base pair DNA size ladder was run in the lanes flanking the test samples.



Capillary Electrophoresis Detection (ABI)

The data shown was generated using the master mix indicated. Amplified products were run on an ABI 3100 instrument. For the master mix: Panel 1 displays data generated testing an alternative 100% clonal control DNA; panel 2 displays data generated testing the recommended 100% clonal control DNA; panel 3 displays data generated testing a 10% dilution of the recommended clonal control DNA; and, panel 4 displays data generated testing the IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). Our IVS-0000 DNA is often tested to provide information regarding valid size ranges for each master mix.



Ordering Information

Catalog #	Products	Quantity
1-101-0020	*IGH* Gene Clonality Assay - Gel Detection	33 reactions
1-101-0040	*IGH* Gene Clonality Assay MegaKit - Gel Detection	330 reactions
1-101-0061	*IGH* Gene Clonality Assay - ABI Fluorescence Detection	33 reactions
1-101-0081	*IGH* Gene Clonality Assay MegaKit - ABI Fluorescence Detection	330 reactions

IGK Gene Clonality Assays

Assay Use

IGK Gene Clonality Assays are useful for studies involving:

- Identification of clonal B-cell populations highly suggestive of B-cell malignancies
- Lineage determination of leukemias and lymphomas
- Monitoring and evaluation of disease recurrence
- Detection and assessment of residual disease
- Evaluation of new research and methods in malignancy studies

Summary and Explanation of the Test

Two master mixes target conserved regions within the variable (Vκ1-7) and the joining (Jκ1-5) regions that flank the unique hypervariable, antigen-binding, complementarity determining region 3 (CDR3). Other primers target the Kde and intragenic regions.

Tube A contains six upstream primers and two Jκ region primers. Tube B contains six upstream Vκ region primers, an upstream intragenic primer and a downstream Kde primer. Positive and negative controls, as well as a Specimen Control Size Ladder Master Mix, are included. PCR products can be analyzed by capillary electrophoresis or heteroduplex analysis. Clonality is indicated if any one of the master mixes generates clonal products.

Background

The human immunoglobulin kappa (*IGK*) light chain locus on the short arm of chromosome 2 (2p12, formerly 2p11.2) spans 1820 kb. It is made up of 76 variable (Vκ) gene segments belonging to 7 subgroups, 5 joining (Jκ) gene segments, and one constant (Cκ) gene segment. Productive assembly of the kappa gene is successful in about 60% of human B lymphocytes[1]. However, even when unsuccessful, clonal B cells generally retain the rearranged kappa genes. The Vκ segments encode the first 95 N-terminal amino acids. Positions 96-108 are encoded by one of five joining (Jκ) gene segments. The constant (Cκ) portion of the kappa light chain (amino acids 109-214) is encoded by a single constant (Cκ) region separated from the Jκ region by an intron. The length of the hypervariable complementarity determining region 3 (CDR3) in kappa light chain genes is limited and rearrangements in this region display significant skewing (platykurtosis)[2].

Therefore, clonal CDR3 products generated from this region are most easily and reliably identified by heteroduplex analysis using standard polyacrylamide gels. Alternatively, capillary electrophoresis or gene sequencing instruments coupled with differential fluorescence detection can be used for analysis. The performance characteristics of this assay have been independently determined by the EuroClonality/BIOMED-2 Group. This consortium of 47 molecular diagnostic laboratories tested hundreds of clinical samples, taking into account their lymphoproliferative status as defined by the WHO classifications[3].

Specimen Requirements

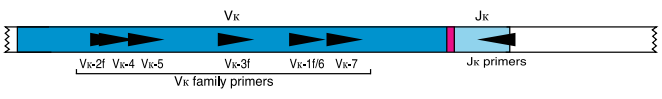
1. 5 mL of peripheral blood, bone marrow biopsy, or bone marrow aspirate anti-coagulated with heparin or EDTA; or,
2. Minimum 5 mm cube of tissue; or,
3. 2 µg of genomic DNA; or,
4. Formalin-fixed, paraffin-embedded (FFPE) tissue or slides.

Reference

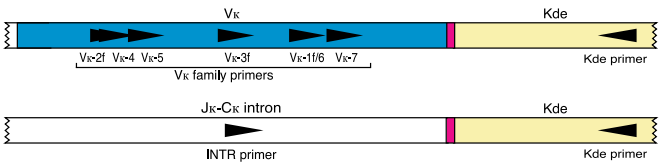
1. AW Langerak et al., *Leukemia* 17: 2275-2280 (2003).
2. EP Rock, PR Sibbald, MM Davis, and YH Chien. *J. Exp. Med.* 179(1): 323-328 (1994).
3. JJM van Dongen et al., *Leukemia* 17: 2257-2317 (2003).

EuroClonality **This assay is based on the EuroClonality/BIOMED-2 Concerted Action BMH4-CT98-3936.**

Figure Legend: Schematic diagram of the immunoglobulin kappa light chain gene complex on chromosome 2p11.2. Shown are the relative positions and orientations for the Vκ-Jκ, and Kde primers, which are included in the *IGK* master mix tubes.



IGK tube A: 6 Vκ primers + 2 Jκ primers



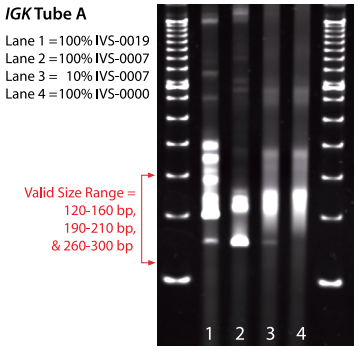
IGK tube B: 6 Vκ primers and INTR primer + 1 Kde primer

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0007 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0000 Polyclonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	**Target**	**Units in Assay**	**Units in Assay MegaKit**
IGK Tube A	Vκ-Jκ	1 x 1500 µL tube	10 x 1500 µL tubes
IGK Tube B	Vκ-Kde	1 x 1500 µL tube	10 x 1500 µL tubes
Specimen Control Size Ladder	Multiple Genes	1 x 1500 µL tube	10 x 1500 µL tubes

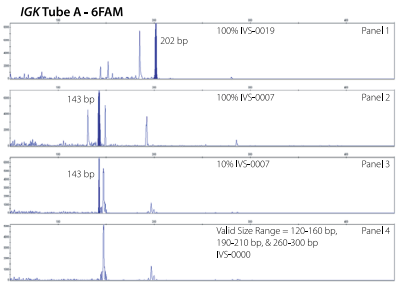
Polyacrylamide Gel Electrophoresis Detection

The data shown was generated using the master mix indicated. Amplified products were heteroduplexed and then run on a 6% polyacrylamide/TBE gel. Lane 1 is data generated testing an alternative 100% clonal control DNA; lane 2 is data generated testing the recommended 100% clonal control DNA; lane 3 is data generated testing a 10% dilution of the recommended clonal control DNA; and, lane 4 is data generated testing IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). The IVS-0000 control is used as a negative control for many of our tests. A standard 100 base pair DNA size ladder was run in the lanes flanking the test samples.



Capillary Electrophoresis Detection (ABI)

The data shown was generated using the master mix indicated. Amplified products were run on an ABI 3100 instrument. Panel 1 displays data generated testing an alternative 100% clonal control DNA; panel 2 displays data generated testing the recommended 100% clonal control DNA; panel 3 displays data generated testing a 10% dilution of the recommended clonal control DNA; and, panel 4 displays data generated testing the IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). Our IVS-0000 DNA is often tested to provide information regarding valid size ranges for each master mix.



Ordering Information

Catalog #	Products	Quantity
1-102-0020	*IGK* Gene Clonality Assay - Gel Detection	33 reactions
1-102-0030	*IGK* Gene Clonality Assay MegaKit - Gel Detection	330 reactions
1-102-0021	*IGK* Gene Clonality Assay - ABI Fluorescence Detection	33 reactions
1-102-0031	*IGK* Gene Clonality Assay MegaKit - ABI Fluorescence Detection	330 reactions

IGL Gene Clonality Assays

Assay Use

IGL Gene Clonality Assays are useful for studies involving:

- Identification of clonal B-cell populations highly suggestive of B-cell malignancies
- Lineage determination of leukemias and lymphomas
- Monitoring and evaluation of disease recurrence
- Detection and assessment of residual disease
- Evaluation of new research and methods in malignancy studies

Summary and Explanation of the Test

The *IGL* Tube master mix targets conserved regions within the variable (Vλ1-3) and the joining (Jλ1-3) regions that flank the unique, hypervariable, antigen-binding, complementarity determining region 3 (CDR3). Positive and negative controls, as well as a Specimen Control Size Ladder Master Mix, are included. PCR products can be analyzed by capillary electrophoresis or heteroduplex analysis. Clonality is indicated if the master mix generates clonal products.

Background

The human immunoglobulin lambda (*IGL*) light chain locus is located on the long arm of chromosome 22 (22q11.2) and spans 1050 kilobases. It is made up of 73-74 variable (Vλ) gene segments (spread over 900 kilobases), 7-11 joining (Jλ) gene segments and 7-11 constant (Cλ) gene segments depending on the haplotypes. Of the 73-74 Vλ region genes, only 30-33 are functional and can be grouped into 11 families and 3 clans[1]. The Jλ and Cλ region genes are organized in tandem with a Jλ segment preceding a Cλ gene. Typically there are 7 Jλ-Cλ segments of which 4 are functional and encode the 4 Ig lambda isotypes.

IGL gene rearrangements are present in 5-10% of Ig kappa B-cell malignancies and in all Ig lambda B-cell malignancies. Therefore, Vλ-Jλ rearrangements potentially represent an attractive extra PCR target for clonality studies to compensate for false-negative *IGH* VH-JH PCR results mainly caused by somatic hypermutations. It should be noted that because of the limited size of the junctional region, it is extremely difficult to distinguish polyclonal from monoclonal rearrangements by running a simple agarose or polyacrylamide gel[1]. Therefore, clonal Vλ-Jλ PCR products are most easily and reliably identified by heteroduplex analysis using standard polyacrylamide gels. Alternatively, capillary electrophoresis or gene sequencing instruments coupled with differential fluorescence detection can be used for analysis[1]. The performance characteristics of this assay have been independently determined by the EuroClonality/BIOMED-2 Group. This consortium of 47 molecular diagnostic laboratories tested hundreds of clinical samples, taking into account their lymphoproliferative status as defined by the WHO classifications[2].

Specimen Requirements

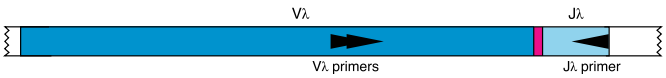
1. 5 mL of peripheral blood, bone marrow biopsy, or bone marrow aspirate anti-coagulated with heparin or EDTA; or,
2. Minimum 5 mm cube of tissue; or,
3. 2 µg of genomic DNA; or,
4. Formalin-fixed, paraffin-embedded (FFPE) tissue or slides.

Reference

1. F Davi et al., *Leukemia* 17:2280-2283 (2003).
2. JJM van Dongen et al., *Leukemia* 17:2257-2317 (2003).

EuroClonality This assay is based on the **EuroClonality/BIOMED-2** Concerted Action BMH4-CT98-3936.

Figure Legend: Schematic diagram of the immunoglobulin lambda light chain gene complex on chromosome 22q11.2. Shown are the relative positions and orientations for the Vλ and Jλ primers, which are included in the *IGL* master mix tube. The two Vλ primers only target Vλ1, 2, and 3 because these three V families cover approximately 70% of rearrangeable Vλ gene segments, and approximately 90% of all *IGL* gene rearrangements involve these three families. Similarly, the single Jλ primer only targets Jλ1, 2, and 3 because these three J segments are involved in 98% of all *IGL* gene rearrangements.



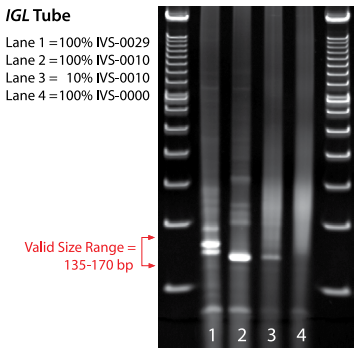
IGL tube: 2 Vλ primers + 1 Jλ primer

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0010 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0029 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0000 Polyclonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	**Target**	**Units in Assay**	**Units in Assay MegaKit**
IGL Tube	VJ	1 x 1500 µL tube	10 x 1500 µL tubes
Specimen Control Size Ladder	Multiple Genes	1 x 1500 µL tube	10 x 1500 µL tubes

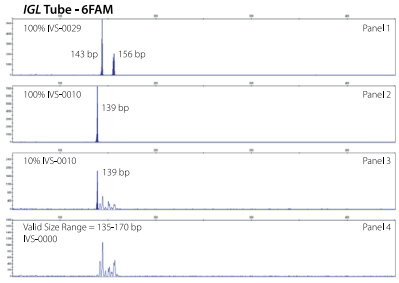
Polyacrylamide Gel Electrophoresis Detection

The data shown was generated using the master mix indicated. Amplified products were heteroduplexed and then run on a 6% polyacrylamide/TBE gel. Lane 1 is data generated testing an alternative 100% clonal control DNA; lane 2 is data generated testing the recommended 100% clonal control DNA; lane 3 is data generated testing a 10% dilution of the recommended clonal control DNA; and, lane 4 is data generated testing IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). The IVS-0000 control is used as a negative control for many of our tests. A standard 100 base pair DNA size ladder was run in the lanes flanking the test samples.



Capillary Electrophoresis Detection (ABI)

The data shown was generated using the master mix indicated. Amplified products were run on an ABI 3100 instrument. Panel 1 displays data generated testing an alternative 100% clonal control DNA; panel 2 displays data generated testing the recommended 100% clonal control DNA; panel 3 displays data generated testing a 10% dilution of the recommended clonal control DNA; and, panel 4 displays data generated testing the IVS-000 Polyclonal Control DNA (Cat# 4-092-0010). Our IVS-0000 DNA is often tested to provide information regarding valid size ranges for each master mix.



Ordering Information

Catalog #	Products	Quantity
1-103-0010	*IGL* Gene Clonality Assay - Gel Detection	33 reactions
1-103-0020	*IGL* Gene Clonality Assay MegaKit - Gel Detection	330 reactions
1-103-0011	*IGL* Gene Clonality Assay - ABI Fluorescence Detection	33 reactions
1-103-0021	*IGL* Gene Clonality Assay MegaKit - ABI Fluorescence Detection	330 reactions

TCRB + *TCRG* T-Cell Clonality Assays

Assay Use

TCRB + *TCRG* T-Cell Clonality Assays are useful for studies involving:

- Identification of clonal T-cell populations highly suggestive of T-cell malignancies
- Lineage determination of leukemias and lymphomas
- Monitoring and evaluation of disease recurrence
- Detection and assessment of residual disease
- Evaluation of new research and methods in malignancy studies

Summary and Explanation of the Test

Five master mixes are included in these test kits to test for rearrangements of both *TRB* (formerly known as *TCRB*) and *TRG* (formerly known as *TCRG*). *TCRB* Tubes A and B target framework regions within the variable region, and the joining region of the T-cell receptor beta locus. *TCRB* Tube C targets the diversity and joining regions. *TCRG* Tubes A and B target framework regions within the variable region, and the joining region of the T-cell receptor gamma locus.

Positive and negative controls, as well as a Specimen Control Size Ladder Master Mix, are included. PCR products can be analyzed by capillary electrophoresis or heteroduplex analysis. Clonality is indicated if any one of the master mixes generates clonal products.

Background

The human T-cell receptor beta (*TRB*, formerly known as *TCRB*) gene locus on chromosome 7 (7q34, formerly 7q35) includes 64-67 variable (Vβ) gene segments (belonging to 30 subgroups), 2 diversity (Dβ) gene segments, and 13 joining (Jβ) gene segments, spread over 685 kilobases. The diversity of this locus has complicated PCR-based testing, however, this standardized multiplex PCR assay detects the vast majority of clonal *TRB* gene rearrangements using only three multiplex master mixes. The detection rate of clonal *TRB* gene rearrangements using this assay is exceptionally high[1].

The T-cell receptor gamma (*TRG*, formerly known as *TCRG*) chain locus spans 128 kb on chromosome 7 (7p14). Rearrangement of the variable (Vγ) and joining (Jγ) genetic segments of the *TRG* locus result in Vγ-Jγ products of unique length and sequence. The *TRG* locus does not contain D segments[2]. In addition, the *TRG* gene contains a limited number of Vγ and Jγ segments such that the amplification of all major Vγ-Jγ combinations is possible with four Vγ and two Jγ primers. This standardized multiplex PCR assay detects the vast majority of clonal *TRG* gene rearrangements using two multiplex master mixes[3]. Using this assay, the clonal *TRG* gene rearrangement detection rate is exceptionally high[3].

PCR products generated from the *TRB* and *TRG* assays are easily and reliably identified by heteroduplex analysis or capillary electrophoresis. The performance characteristics of this assay have been independently determined by the EuroClonality/BIOMED-2 Group. This consortium of 47 molecular diagnostic laboratories tested hundreds of clinical samples, taking into account their lymphoproliferative status as defined by the WHO classifications[2].

Specimen Requirements

1. 5 mL of peripheral blood, bone marrow biopsy, or bone marrow aspirate anti-coagulated with heparin or EDTA; or,
2. Minimum 5 mm cube of tissue; or,
3. 2 µg of genomic DNA; or,
4. Formalin-fixed, paraffin-embedded (FFPE) tissue or slides.

Reference

1. M Brüggemann et al., *Leukemia* 17: 2283-2289 (2003).
2. JJM van Dongen et al., *Leukemia* 17: 2257-2317 (2003).
3. K Beldjord et al., *Leukemia* 17: 2289-2292 (2003).

EuroClonality

This assay is based on the EuroClonality/BIOMED-2 Concerted Action BMH4-CT98-3936.

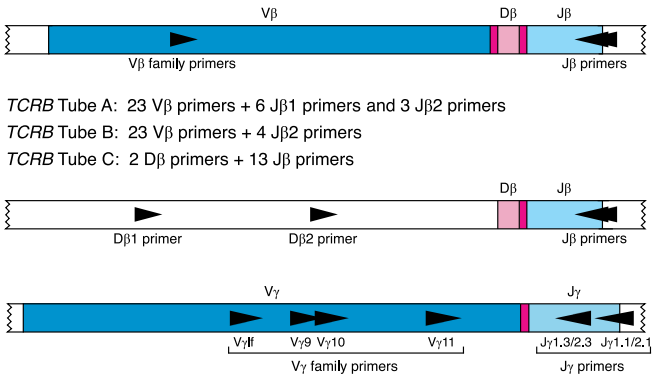


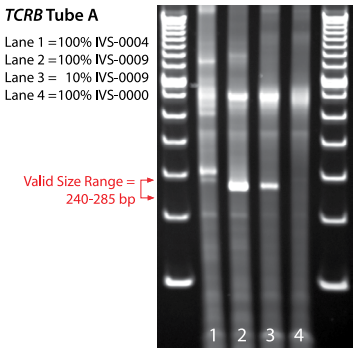
Figure Legend: Simplified diagram of a representative rearranged T-cell receptor beta gene and the T-cell receptor gamma gene showing the approximate placement of the upstream and downstream DNA primers. The numbers of primers and their specificity are listed for master mix *TCRB* Tubes A, B, and C and *TCRG* Tubes A and B. (The VγIf primer is a consensus primer that targets Vγ1 through Vγ8).

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0009 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0004 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0021 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0000 Polyclonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	**Target**	**Units in Assay**	**Units in Assay MegaKit**
TCRB Tube A	Multiple Vβ + Jβ1/2	1 x 1500 µL tube	10 x 1500 µL tubes
TCRB Tube B	Multiple Vβ + Jβ2	1 x 1500 µL tube	10 x 1500 µL tubes
TCRB Tube C	Multiple Dβ + Jβ1/2	1 x 1500 µL tube	10 x 1500 µL tubes
TCRG Tube A	Vγ1-8 + Vγ10 + Jγ	1 x 1500 µL tube	10 x 1500 µL tubes
TCRG Tube B	Vγ9+ Vγ11 + Jγ	1 x 1500 µL tube	10 x 1500 µL tubes
Specimen Control Size Ladder	Multiple Genes	1 x 1500 µL tube	10 x 1500 µL tubes

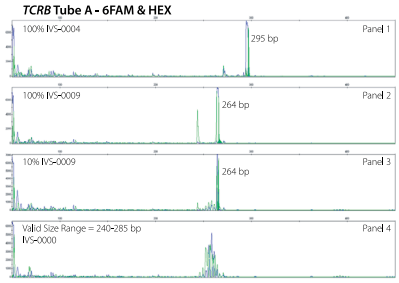
Polyacrylamide Gel Electrophoresis Detection

The data shown was generated using the master mix indicated. Amplified products were heteroduplexed and run on a 6% polyacrylamide/TBE gel. Lane 1 is data generated testing an alternative 100% clonal control DNA; lane 2 is data generated testing the recommended 100% clonal control DNA; lane 3 is data generated testing a 10% dilution of the recommended clonal control DNA; and, lane 4 is data generated testing IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). The IVS-0000 control is used as a negative control for many of our tests. A standard 100 base pair DNA size ladder was run in the lanes flanking the test samples.



Capillary Electrophoresis Detection (ABI)

The data shown was generated using the master mix indicated. Amplified products were run on an ABI 3100 instrument. Panel 1 displays data generated testing an alternative 100% clonal control DNA; panel 2 displays data generated testing the recommended 100% clonal control DNA; panel 3 displays data generated testing a 10% dilution of the recommended clonal control DNA; and, panel 4 displays data generated testing the IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). Our IVS-0000 DNA is often tested to provide information regarding valid size ranges for the master mix.



Ordering Information

Catalog #	Products	Quantity
1-200-0010	*TCRB* + *TCRG* T-Cell Clonality Assay - Gel Detection	33 reactions
1-200-0020	*TCRB* + *TCRG* T-Cell Clonality Assay MegaKit - Gel Detection	330 reactions
1-200-0011	*TCRB* + *TCRG* T-Cell Clonality Assay - ABI Fluorescence Detection	33 reactions
1-200-0021	*TCRB* + *TCRG* T-Cell Clonality Assay MegaKit - ABI Fluorescence Detection	330 reactions

TCRB Gene Clonality Assays

Assay Use

TCRB Gene Clonality Assays are useful for studies involving:

- Identification clonal T-cell populations highly suggestive of T-cell malignancies
- Lineage determination of leukemias and lymphomas
- Monitoring and evaluation of disease recurrence
- Detection and assessment of residual disease
- Evaluation of new research and methods in malignancy studies

Summary and Explanation of the Test

Three multiplex master mixes target conserved regions within the variable (Vβ), diversity (Dβ), and the joining (Jβ) regions that flank the unique hypervariable, antigen-binding, complementarity determining region 3 (CDR3) of the T-cell receptor beta locus. Tube A contains 23 Vβ primers, 6 Jβ1 primers, and 3 Jβ2 primers. Tube B contains 23 Vβ and 4 Jβ2 primers. Tube C contains 2 Dβ and 13 Jβ primers. Positive and negative DNA controls, as well as a Specimen Control Size Ladder Master Mix, are included. PCR products can be analyzed by capillary electrophoresis or heteroduplex analysis. Clonality is indicated, if any one of the master mixes generates clonal products.

Background

The human T-cell receptor beta (*TRB*, formerly known as *TCRB*) gene locus on chromosome 7 (7q34, formerly 7q35) includes 64-67 variable (Vβ) gene segments (belonging to 30 subgroups), 2 diversity (Dβ) gene segments, and 13 joining (Jβ) gene segments, spread over 685 kilobases. The diversity of this locus has complicated PCR-based testing and extended dependence on Southern blot analysis in many testing centers. However, this standardized multiplex PCR assay detects the vast majority of clonal *TRB* gene rearrangements using only three multiplex master mixes[1].

This assay provides rapid *TCR* clonality assessment, reducing or completely eliminating the number of Southern blot tests performed in the laboratory. The detection rate of clonal *TRB* gene rearrangements using this assay is exceptionally high[1]. The performance characteristics of this assay have been independently determined by the EuroClonality/BIOMED-2 Group. This consortium of 47 molecular diagnostic laboratories tested hundreds of clinical samples, taking into account their lymphoproliferative status as defined by the WHO classifications[2].

Specimen Requirements

1. 5 mL of peripheral blood, bone marrow biopsy, or bone marrow aspirate anti-coagulated with heparin or EDTA; or,
2. Minimum 5 mm cube of tissue; or,
3. 2 µg of genomic DNA; or,
4. Formalin-fixed, paraffin-embedded (FFPE) tissue or slides.

Reference

1. M Brüggemann et al., *Leukemia* 17: 2283-2289 (2003).
2. JJM van Dongen et al., *Leukemia* 17: 2257-2317 (2003).

EuroClonality This assay is based on the **EuroClonality/BIOMED-2** Concerted Action BMH4-CT98-3936.

Figure Legend: Simplified diagram of a representative rearranged T-cell receptor beta gene showing the approximate placement of the upstream and downstream DNA primers. The numbers of primers and their specificity are listed for Master Mix Tubes A, B, and C.

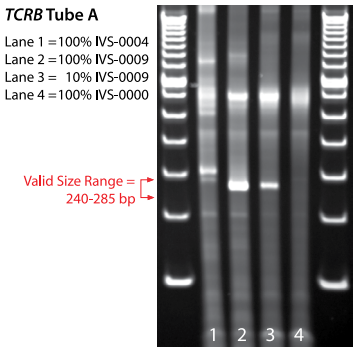
Tube A: 23 Vβ primers + 6 Jβ1 primers and 3 Jβ2 primers
Tube B: 23 Vβ primers + 4 Jβ2 primers
Tube C: 2 Dβ primers + 13 Jβ primers

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0009 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0004 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0000 Polyclonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	**Target**	**Units in Assay**	**Units in Assay MegaKit**
TCRB Tube A	Multiple Vβ + Jβ1/2	1 x 1500 µL tube	10 x 1500 µL tubes
TCRB Tube B	Multiple Vβ + Jβ2	1 x 1500 µL tube	10 x 1500 µL tubes
TCRB Tube C	Multiple Dβ + Jβ1/2	1 x 1500 µL tube	10 x 1500 µL tubes
Specimen Control Size Ladder	Multiple Genes	1 x 1500 µL tube	10 x 1500 µL tubes

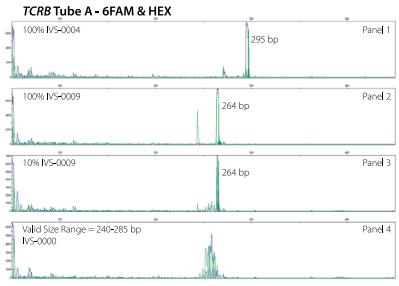
Polyacrylamide Gel Electrophoresis Detection

The data shown was generated using the master mix indicated. Amplified products were heteroduplexed and run on a 6% polyacrylamide/TBE gel. Lane 1 is data generated testing an alternative 100% clonal control DNA; lane 2 is data generated testing the recommended 100% clonal control DNA; lane 3 is data generated testing a 10% dilution of the recommended clonal control DNA; and, lane 4 is data generated testing IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). The IVS-0000 control is used as a negative control for many of our tests. A standard 100 base pair DNA size ladder was run in the lanes flanking the test samples.



Capillary Electrophoresis Detection (ABI)

The data shown was generated using the master mix indicated. Amplified products were run on an ABI 3100 instrument. Panel 1 displays data generated testing an alternative 100% clonal control DNA; panel 2 displays data generated testing the recommended 100% clonal control DNA; panel 3 displays data generated testing a 10% dilution of the recommended clonal control DNA; and, panel 4 displays data generated testing the IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). Our IVS-0000 DNA is often tested to provide information regarding valid size ranges for each master mix.



Ordering Information

Catalog #	Products	Quantity
1-205-0010	*TCRB* Gene Clonality Assay - Gel Detection	33 reactions
1-205-0020	*TCRB* Gene Clonality Assay MegaKit - Gel Detection	330 reactions
1-205-0011	*TCRB* Gene Clonality Assay - ABI Fluorescence Detection	33 reactions
1-205-0021	*TCRB* Gene Clonality Assay MegaKit - ABI Fluorescence Detection	330 reactions

T-Cell Receptor Gamma Gene Rearrangement Assay 2.0

Assay Use

T-Cell Receptor Gamma Gene Rearrangement Assays are useful for studies involving:

- Identification of clonal T-cell populations highly suggestive of T-cell malignancies
- Monitoring and evaluation of disease recurrence
- Evaluation of new research and methods in malignancy studies

Summary and Explanation of the Test

This T-Cell Receptor Gamma Gene Rearrangement Assay represents an improved approach to PCR-based clonality testing of lymphoproliferative disorders, as it can detect the vast majority of *TCR* gamma gene rearrangements with a single multiplex master mix. Importantly, this assay includes, in a single tube, primers for all known groups of *TCR* gamma variable (Vγ) region genes and joining (Jγ) region genes that are involved in rearrangements of T-cell lymphomas. In addition, all reverse primers that target the Jγ region genes are conjugated with the 6FAM fluorophore. Positive and negative controls, as well as a Specimen Control Size Ladder Master Mix are included. PCR products are analyzed by capillary electrophoresis.

Background

The human T-cell receptor gamma (*TRG*, formerly known as *TCRG*) gene locus on chromosome 7 (7q14) includes 14 Vγ genes belonging to 4 subgroups, 5 Jγ segments, and 2 Cγ genes spread over 200 kilobases. The diversity of this locus has historically complicated PCR-based testing. Our new multiplex PCR assay represents an improvement over existing assays as it can detect the vast majority of *TCR* gamma gene rearrangements with a single multiplex master mix. This master mix targets all conserved regions within the variable (Vγ) and joining (Jγ) region genes that are described in lymphoid malignancies. This is critical for more comprehensive analysis of patient samples, as some T-cell lymphoproliferative disorders involve Vγ and Jγ regions that would not be identified with a single Vγ(1-8) and Jγ1/Jγ2 primer set.

In addition, the polyclonal background that results from the combination of all primers in a single tube produces a more robust and easily interpreted signal with capillary electrophoresis, which aids in the interpretation of small peaks. Competitive amplification of all *TRG* gene rearrangements allows for identification of a quantitative threshold for a positive result and helps to avoid false positive results. The average size of the *TRG* gene rearrangement PCR amplicons is 190 nucleotides, with a normal distribution of product sizes between 159 and 207 nucleotides. This protocol should lead to improved product formation from formalin-fixed, paraffin-embedded (FFPE) samples compared to other protocols that yield products of 260 nucleotides or larger.

Specimen Requirements

1. 5 mL of peripheral blood, bone marrow biopsy, or bone marrow aspirate anti-coagulated with heparin or EDTA; or,
2. Minimum 5 mm cube of tissue; or,
3. 2 µg of genomic DNA; or,
4. Formalin-fixed, paraffin-embedded tissue or slides.

Reference

1. TC Greiner et al., *JMD* 4: 137-143 (2002).
2. LC Lawnickie et al., *JMD* 5: 82-87 (2003).
3. Y Sandberg et al., *Leukemia* 21: 21 (2007).

EuroClonality **This assay was developed by Invivoscribe. The performance of this assay was reviewed and validated by the EuroClonality/ BIOMED-2 Group. Euroclonality manuscript in preparation: multicenter study with 250 clinical patient specimens.**

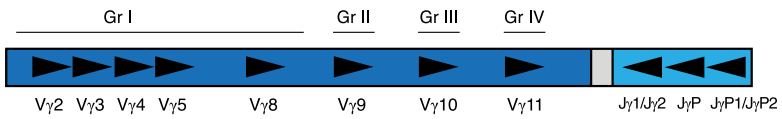


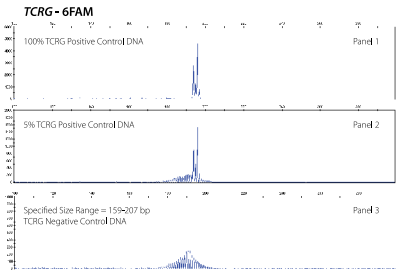
Figure Legend: Simple representation of the organization of the T-cell receptor gamma gene on chromosome 7. Black arrows represent the relative positions of primers that target the variable region genes and the downstream joining region gene segments that are involved in rearrangements in T-cell lymphomas. The downstream primers are fluorescently labeled through the incorporation of a 6FAM fluorophore. The amplicon products generated from these rearrangements are detected by capillary electrophoresis.

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
5% *TCRG* Positive Control DNA	50 µg/mL	1 x 50 µL tube	5 x 50 µL tube
TCRG Negative Control DNA	50 µg/mL	1 x 50 µL tube	5 x 50 µL tube
Master Mixes	**Target**	**Units in Assay**	**Units in Assay MegaKit**
TCRG - 6FAM	Vγ1-Vγ11 + Jγ1/Jγ2, Jγ P, Jγ P1/Jγ P2	1 x 1500 µL tube	10 x 1500 µL tubes
Specimen Control Size Ladder	Multiple Genes	1 x 1500 µL tube	10 x 1500 µL tubes

Capillary Electrophoresis Detection (ABI)

The data shown was generated using the *TCRG*-6FAM master mix. Amplified products were run on a capillary electrophoresis ABI 3130xl instrument. Panel 1 displays data generated testing 100% *TCRG* Positive Control DNA (DNA isolated from a cell line known to have both a Vγ9 + Jγ1/Jγ2 and a Vγ10 + Jγ1/Jγ2 rearrangement); panel 2 displays data generated from the testing of the 5% *TCRG* Positive Control DNA; and, panel 3 displays data generated from the testing of the polyclonal *TCRG* Negative Control DNA.



Ordering Information

Catalog #	Products	Quantity
1-207-0101	T-Cell Receptor Gamma Gene Rearrangement Assay 2.0 - ABI Fluorescence Detection	33 reactions
1-207-0111	T-Cell Receptor Gamma Gene Rearrangement Assay MegaKit 2.0 - ABI Fluorescence Detection	330 reactions

T-Cell Receptor Gamma Gene Rearrangement Assays

Assay Use

T-Cell Receptor Gamma Gene Rearrangement Assays are useful for studies involving:

- Identification of clonal T-cell populations highly suggestive of T-cell malignancies
- Lineage determination of leukemias and lymphomas
- Monitoring and evaluation of disease recurrence
- Detection and assessment of residual disease
- Evaluation of new research and methods in malignancy studies

Summary and Explanation of the Test

Sample genomic DNA is amplified using two master mixes that independently target conserved regions within the variable (Vγ) and joining (Jγ) regions that flank the unique, hypervariable, antigen-binding, complementarity determining region 3 (CDR3). This assay targets Vγ1-9 and Jγ gene segments. Positive and negative DNA controls, as well as an internal Amplification Control Master Mix, are included. The limit of detection of this assay is approximately one clonal T cell in a background of a hundred normal cells. PCR products can be analyzed by capillary electrophoresis or standard gel electrophoresis with ethidium bromide staining.

Background

The T-cell receptor gamma (*TRG*, formerly known as *TCRG*) chain locus spans 160 kilobases on chromosome 7 (7p14). The locus consists of 14 variable (Vγ) gene segments in 6 subgroups, and 5 joining (Jγ) gene segments interspersed between 2 constant (Cγ) gene segments. However, the repertoire of functional TRG molecules is limited to 4-6 functional Vγ gene segments that belong to 2 subgroups[2].

Rearrangement of the Vγ and Jγ gene segments of the *TRG* locus results in Vγ-Jγ products of unique length and sequence. Clonal *TRG* rearrangements can be most rapidly identified by analyzing the size distribution of DNA products amplified from conserved sequences that flank this Vγ-Jγ region[1]. DNA isolated from a normal heterogeneous population of polyclonal T-cells produces a Gaussian distribution (bell-shaped size curve) of amplified products. DNA amplified from a clonal T-cell population generates one or two product(s) of unique size that reflects proliferation of a single rearranged clone[1,2].

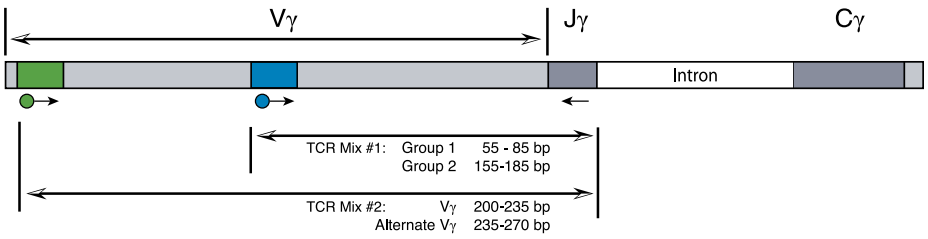
Specimen Requirements

1. 5 mL of peripheral blood, bone marrow biopsy, or bone marrow aspirate anti-coagulated with heparin or EDTA; or,
2. Minimum 5 mm cube of tissue; or,
3. 2 µg of genomic DNA; or,
4. Formalin-fixed, paraffin-embedded (FFPE) tissue or slides.

Reference

1. JE Miller, SS Wilson, DL Jaye, and M Kronenberg. *J. Mol. Diag.* 4: 101-117 (1999).
2. K Beldjord et al., *Leukemia* 17: 2289-2292 (2003).

Figure Legend: Simplified figure representing the organization of a rearranged T-cell receptor gamma chain gene on chromosome 7. Colored arrows represent conserved regions within the variable region gene segments targeted by primers. Primers are represented by arrows with the size range of valid products generated with each of the master mixes indicated below the figure. Colors correspond to the peak colors assigned to products when differential fluorescence detection methods are used.

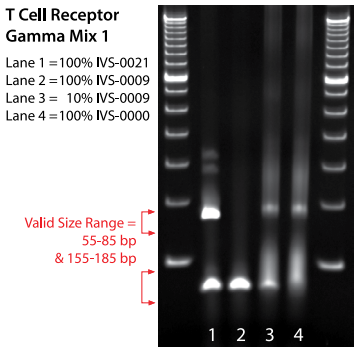


Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0009 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0000 Polyclonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	**Target**	**Units in Assay**	**Units in Assay MegaKit**
T-Cell Receptor Gamma Mix 1	Vγ1-8,9 + Jγ1/2	1 x 1500 µL tube	10 x 1500 µL tubes
T-Cell Receptor Gamma Mix 2	Alt Vγ + Jγ1/2	1 x 1500 µL tube	10 x 1500 µL tubes
Amplification Control	*HLA-DQa*	1 x 1500 µL tube	10 x 1500 µL tubes

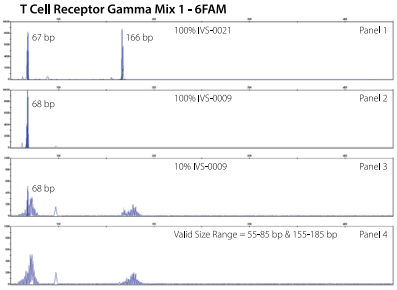
Polyacrylamide Gel Electrophoresis Detection

The data shown was generated using the master mix indicated. Amplified products were run on a 6% polyacrylamide/TBE gel. Lane 1 is data generated testing an alternative 100% clonal control DNA; lane 2 is data generated testing the recommended 100% clonal control DNA; lane 3 is data generated testing a 10% dilution of the recommended clonal control DNA; and, lane 4 is data generated testing IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). The IVS-0000 control is used as a negative control for many of our tests. A standard 100 base pair DNA size ladder was run in the lanes flanking the test samples.



Capillary Electrophoresis Detection (ABI)

The data shown was generated using the master mix indicated. Amplified products were run on an ABI 3100 instrument. Panel 1 displays data generated testing an alternative 100% clonal control DNA; panel 2 displays data generated testing the recommended 100% clonal control DNA; panel 3 displays data generated testing a 10% dilution of the recommended clonal control DNA; and, panel 4 displays data generated testing the IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). Our IVS-0000 DNA is often tested to provide information regarding valid size ranges for each master mix.



Ordering Information

Catalog #	Products	Quantity
1-207-0010	T-Cell Receptor Gene Rearrangement Assay - Gel Detection	33 reactions
1-207-0030	T-Cell Receptor Gene Rearrangement Assay MegaKit - Gel Detection	330 reactions
1-207-0051	T-Cell Receptor Gene Rearrangement Assay - ABI Fluorescence Detection	33 reactions
1-207-0071	T-Cell Receptor Gene Rearrangement Assay MegaKit - ABI Fluorescence Detection	330 reactions

TCRG Gene Clonality Assays

Assay Use

TRG Gene Clonality Assays are useful for studies involving:

- Identification of clonal T-cell populations highly suggestive of T-cell malignancies
- Lineage determination of leukemias and lymphomas
- Monitoring and evaluation of disease recurrence
- Detection and assessment of residual disease
- Evaluation of new research and methods in malignancy studies

Summary and Explanation of the Test

This assay tests all variable (Vγ) regions 1-11 of the *TRG* (formerly known as *TCRG*) gene.. Master mix tubes A and B target conserved regions within the variable (Vγ) and joining (Jγ) regions that flank the unique, hypervariable, antigen-binding, complementarity determining region 3 (CDR3). Tube A contains two Vγ primers and two Jγ primers. Tube B contains two Vγ primers and two Jγ primers. Positive and negative controls, as well as a Specimen Control Size Ladder Master Mix are included. PCR products can be analyzed by capillary electrophoresis or heteroduplex analysis. Clonality is indicated if any one of the master mixes generates clonal products.

Background

The T-cell receptor gamma (*TRG*, formerly known as *TCRG*) chain locus spans 128 kb on chromosome 7 (7p14). Rearrangement of the variable (Vγ) and joining (Jγ) genetic segments of the *TRG* locus result in Vγ-Jγ products of unique length and sequence. The *TRG* locus does not contain diversity (Dγ) segments[2]. *TRG* is a preferential target for clonality analyses since it is rearranged in greater than 90% of T-ALL, T-large granular lymphocyte (LGL), and T-PLL, in 50-75% of peripheral T-NHL and mycosis fungoides, but not NK cell proliferations. It is also rearranged in a major part (60%) of B-lineage ALLs and in a much smaller part of B NHLs. In addition, the *TRG* gene contains a limited number of Vγ and Jγ segments such that the amplification of all major Vγ-Jγ combinations is possible with four Vγ and two Jγ primers.

This standardized multiplex PCR assay detects the vast majority of clonal *TRG* gene rearrangements using only two multiplex master mixes[1]. The potential risk of false-positive results, due to overinterpretation of minor clonal peaks, can be minimized by the combined use of heteroduplex analysis and differential fluorescence detection, and by interpreting results within their clinical context[1,2].

The detection rate of clonal *TRG* gene rearrangements using this assay is exceptionally high[1]. The performance characteristics of this assay have been independently determined by the EuroClonality/BIOMED-2 Group. This consortium of 47 molecular diagnostic laboratories tested hundreds of clinical samples, taking into account their lymphoproliferative status as defined by the WHO classifications[2].

Specimen Requirements

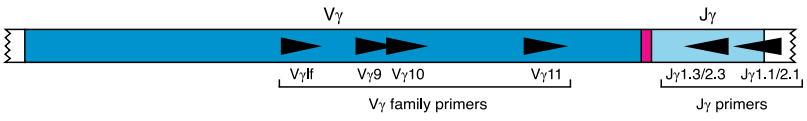
1. 5 mL of peripheral blood, bone marrow biopsy, or bone marrow aspirate anti-coagulated with heparin or EDTA; or,
2. Minimum 5 mm cube of tissue; or,
3. 2 µg of genomic DNA; or,
4. Formalin-fixed, paraffin-embedded (FFPE) tissue or slides.

Reference

1. K Beldjord et al., *Leukemia* 17: 2289-2292 (2003).
2. JJM van Dongen et al., *Leukemia* 17: 2257-2317 (2003).

EuroClonality **This assay is based on the EuroClonality/BIOMED-2 Concerted Action BMH4-CT98-3936.**

Figure Legend: Depicted is a simple representation of the organization of the T-cell receptor gamma chain gene on chromosome 7. Black arrows represent the relative positions of primers that target the variable (V*γ*) regions, and the downstream joining (J*γ*) gene segments. The amplicon products generated from each of these regions can be differentially detected when fluorescent primer sets are used with capillary electrophoresis instruments that employ differential fluorescence detection.



TCRG tube A: Vγlf and Vγ10 primers + Jγ1.1/2.1 and Jγ1.3/2.3

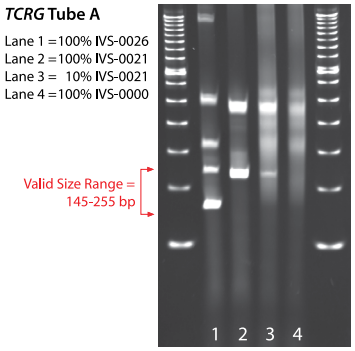
TCRG tube B: Vγ9 and Vγ11 primers + Jγ1.1/2.1 and Jγ1.3/2.3

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0009 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0021 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0000 Polyclonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	**Target**	**Units in Assay**	**Units in Assay MegaKit**
TCRG Tube A	Vγ1-8 + Vγ10 + Jγ	1 x 1500 µL tube	10 x 1500 µL tubes
TCRG Tube B	Vγ9 + Vγ11 + Jγ	1 x 1500 µL tube	10 x 1500 µL tubes
Specimen Control Size Ladder	Multiple Genes	1 x 1500 µL tube	10 x 1500 µL tubes

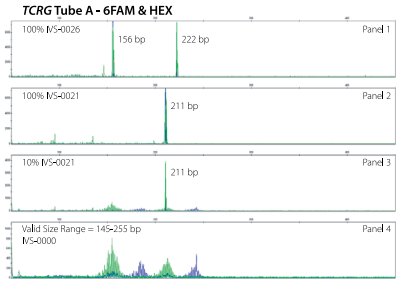
Polyacrylamide Gel Electrophoresis Detection

The data shown was generated using the master mix indicated. Amplified products were heteroduplexed and run on a 6% polyacrylamide/TBE gel. Lane 1 is data generated testing an alternative 100% clonal control DNA; lane 2 is data generated testing the recommended 100% clonal control DNA; lane 3 is data generated testing a 10% dilution of the recommended clonal control DNA; and, lane 4 is data generated testing IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). The IVS-0000 control is used as a negative control for many of our tests. A standard 100 base pair DNA size ladder was run in the lanes flanking the test samples.



Capillary Electrophoresis Detection (ABI)

The data shown was generated using the master mix indicated. Amplified products were run on an ABI 3100 instrument. Panel 1 displays data generated testing an alternative 100% clonal control DNA; panel 2 displays data generated testing the recommended 100% clonal control DNA; panel 3 displays data generated testing a 10% dilution of the recommended clonal control DNA; and, panel 4 displays data generated testing the IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). Our IVS-0000 DNA is often tested to provide information regarding valid size ranges for each master mix.



Ordering Information

Catalog #	Products	Quantity
1-207-0020	*TRG* Gene Clonality Assay - Gel Detection	33 reactions
1-207-0040	*TRG* Gene Clonality Assay MegaKit - Gel Detection	330 reactions
1-207-0021	*TRG* Gene Clonality Assay - ABI Fluorescence Detection	33 reactions
1-207-0041	*TRG* Gene Clonality Assay MegaKit - ABI Fluorescence Detection	330 reactions

TCRD Gene Clonality Assays

Assay Use

TCRD Gene Clonality Assays are useful for studies involving:

- Identification of clonal T-cell populations highly suggestive of T-cell malignancies
- Lineage determination of leukemias and lymphomas
- Monitoring and evaluation of disease recurrence
- Detection and assessment of residual disease
- Evaluation of new research and methods in malignancy studies

Summary and Explanation of the Test

The *TCRD* Tube Master Mix targets conserved regions within the variable (Vδ1-6), the diversity (Dδ2-3) and the joining (Jδ1-4) regions that flank the unique, hypervariable, antigen-binding, complementarity determining region 3 (CDR3) of the T-cell receptor delta (*TRD*, formerly known as *TCRD*). Positive and negative controls, as well as a Specimen Control Size Ladder Master Mix, are included. PCR products can be analyzed by capillary electrophoresis or heteroduplex analysis. Clonality is indicated if the master mix generates clonal products.

Background

The human T-cell receptor delta (*TRD*, formerly known as *TCRD*) gene locus is comprised of a cluster of 10 genes located on chromosome 14 (14q11.2) spread over 60 kilobases, localized between the T-cell receptor alpha (*TRA*, formerly known as *TCRA*) variable (Vα) and joining (Jα) gene segments. It is made up of 8 variable (Vδ), 3 diversity (Dδ), and 4 joining (Jδ) gene segments[1]. At least 5 of the 8 Vδ gene segments can also rearrange to Jδ gene segments and other Vδ gene segments may also be utilized in *TRD* gene rearrangements in rare cases. Although the small number of Vδ, Dδ, and Jδ gene segments available for recombination limits the potential combinatorial diversity, the complementarity determining region 3 (CDR3) or junctional diversity is extensive due to the addition of N regions, P regions, and random deletion of nucleotides by recombinases. This diversity is also extended by the recombination of up to three Dδ segments and therefore up to four N regions within the rearranged *TRD* locus. This limited germline diversity encoded at the *TRD* locus in conjunction with extensive junctional diversity results in a useful target for PCR analysis. *TRD* recombination events have been used most extensively as clonal markers in both T- and B-cell ALL. This standardized multiplex PCR assay detects the vast majority of clonal *TRD* gene rearrangements using a single multiplex master mix[1]. This assay provides rapid *TCR* clonality assessment, reducing or eliminating the number of Southern blot tests performed in the laboratory[2]. The detection rate of clonal *TRD* gene rearrangements using this assay is exceptionally high[1]. The performance characteristics of this assay have been independently determined by the EuroClonality/BIOMED-2 Group. This consortium of 47 molecular diagnostic laboratories tested hundreds of clinical samples, taking into account their lymphoproliferative status as defined by the WHO classifications[2].

Specimen Requirements

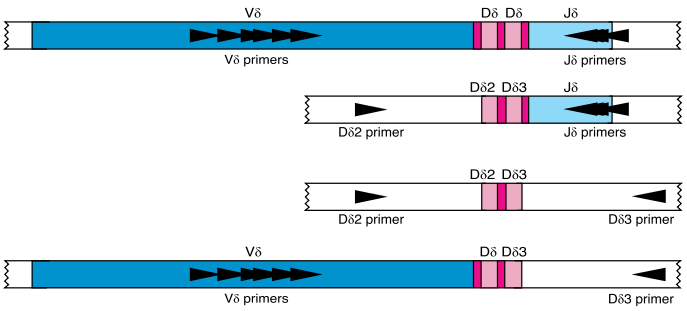
1. 5 mL of peripheral blood, bone marrow biopsy, or bone marrow aspirate anti-coagulated with heparin or EDTA; or,
2. Minimum 5 mm cube of tissue; or,
3. 2 µg of genomic DNA; or,
4. Formalin-fixed, paraffin-embedded (FFPE) tissue or slides.

Reference

1. FL Lavender et al., *Leukemia* 17: 2292-2296 (2003).
2. JJM van Dongen et al., *Leukemia* 17: 2257-2317 (2003).

EuroClonality **This assay is based on the EuroClonality/BIOMED-2 Concerted Action BMH4-CT98-3936.**

Figure Legend: Simplified diagram of a representative rearranged T-cell receptor delta gene showing the approximate placement of the upstream and downstream DNA primers. The numbers of primers and their specificity are listed for the *TRD* Tube Master Mix tube.



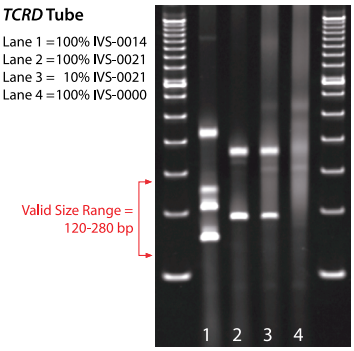
TCRD tube: 6 Vδ and 1 Dδ2 primers + 4 Jδ and 1 Dδ3 primers

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0021 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0000 Polyclonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	**Target**	**Units in Assay**	**Units in Assay MegaKit**
TCRD Tube	Multiple Vδ + Dδ + Jδ	1 x 1500 µL tube	10 x 1500 µL tubes
Specimen Control Size Ladder	Multiple Genes	1 x 1500 µL tube	10 x 1500 µL tubes

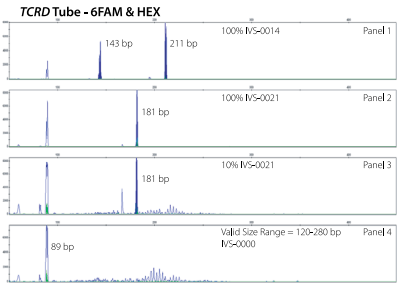
Polyacrylamide Gel Electrophoresis Detection

The data shown was generated using the master mix indicated. Amplified products were heteroduplexed and run on a 6% polyacrylamide/TBE gel. Lane 1 is data generated testing an alternative 100% clonal control DNA; lane 2 is data generated testing the recommended 100% clonal control DNA; lane 3 is data generated testing a 10% dilution of the recommended clonal control DNA; and, lane 4 is data generated testing IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). The IVS-0000 control is used as a negative control for many of our tests. A standard 100 base pair DNA size ladder was run in the lanes flanking the test samples.



Capillary Electrophoresis Detection (ABI)

The data shown was generated using the master mix indicated. Amplified products were run on an ABI 3100 instrument. Panel 1 displays data generated testing an alternative 100% clonal control DNA; panel 2 displays data generated testing the recommended 100% clonal control DNA; panel 3 displays data generated testing a 10% dilution of the recommended clonal control DNA; and, panel 4 displays data generated testing the IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). Our IVS-0000 DNA is often tested to provide information regarding valid size ranges for each master mix.



Ordering Information

Catalog #	Products	Quantity
1-206-0010	*TCRD* Gene Clonality Assay - Gel Detection	33 reactions
1-206-0020	*TCRD* Gene Clonality Assay MegaKit - Gel Detection	330 reactions
1-206-0011	*TCRD* Gene Clonality Assay - ABI Fluorescence Detection	33 reactions
1-206-0021	*TCRD* Gene Clonality Assay MegaKit - ABI Fluorescence Detection	330 reactions

BCL1/JH Translocation Assay

Assay Use

The *BCL1*/JH Translocation Assay is useful for studies involving:

- Identification of *IGH-BCL1* (now known as *IGH-CCND1*) gene rearrangements highly suggestive of mantle cell lymphoma
- Lineage determination of leukemias and lymphomas
- Monitoring and evaluation of disease recurrence
- Detection and assessment of residual disease
- Evaluation of new research and methods in malignancy studies

Summary and Explanation of the Test

Two master mixes are included in this assay kit. The *BCL1*/JH Master Mix targets the major translocation cluster (MTC) of the *CCND1* locus (formerly known as *BCL1*) and the joining region (JH) of the immunoglobulin heavy chain locus (IGH). The Specimen Control Size Ladder Master Mix targets multiple genes and generates a series of amplicons of approximately 100, 200, 300, 400, and 600 base pairs to ensure that the quality and quantity of input DNA is adequate to yield a valid result. Positive and negative controls are included. PCR products can be analyzed using standard gel electrophoresis with ethidium bromide staining. A *CCND1* translocation is indicated if the master mix generates product(s) within the valid size range.

Background

The *IGH-CCND1* t(11;14)(q13;q32) translocation is mainly found in mantle cell lymphomas, but has also been seen in B-prolymphocytic leukemia (10-20%), plasma cell leukemia, splenic lymphoma with villous lymphocytes, chronic lymphocytic leukemia (2-5%), and in multiple myeloma (20-25%). The t(11;14) brings about juxtaposition of the cyclin D1 gene with the immunoglobulin heavy chain gene. This leads to a marked increase in expression of cyclin D1 driven by the Ig heavy chain gene enhancer, located in the intron between the JH and constant region genes. The overexpression of cyclin D1 accelerates the passage of transformed cells through the G1 phase. Approximately 41% of the breakpoints on the *CCND1/MTC* locus can be detected by PCR methodology. However, breakpoints outside of the *CCND1/MTC* locus will not be identified by this particular test. Therefore, a negative result does not completely exclude the presence of an *IGH-CCND1* gene translocation in the sample[1]. The performance characteristics of this assay have been independently determined by the EuroClonality/BIOMED-2 Group. This consortium of 47 molecular diagnostic laboratories tested hundreds of clinical samples, taking into account their lymphoproliferative status as defined by the WHO classifications[2].

Specimen Requirements

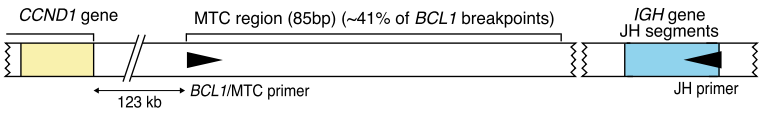
1. 5 mL of peripheral blood, bone marrow biopsy, or bone marrow aspirate anti-coagulated with heparin or EDTA; or,
2. Minimum 5 mm cube of tissue; or,
3. 2 µg of genomic DNA; or,
4. Formalin-fixed, paraffin-embedded (FFPE) tissue or slides.

Reference

1. P Wijers et al., *Leukemia* 17: 2296-2298 (2003).
2. JJM van Dongen et al., *Leukemia* 17: 2257-2317 (2003).

EuroClonality **This assay is based on the EuroClonality/BIOMED-2 Concerted Action BMH4-CT98-3936.**

Figure Legend: Schematic diagram of the *IGH-CCND1* t(11;14) translocation showing the cyclin D1 (*CCND1*) gene on the left and the Ig heavy chain (*IGH*) gene on the right. Shown are the relative positions and orientations for the *BCL1*/MTC primer and the JH primer, which are included in the *BCL1*/JH Master Mix tube.



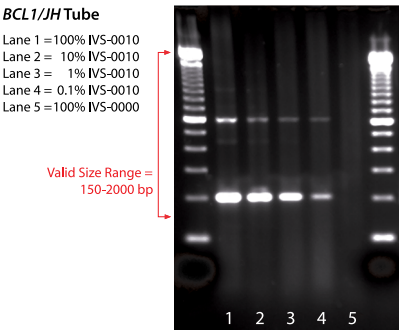
t(11;14) tube: 1 *BCL1* MTC primer + 1 JH primer

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0010 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0000 Polyclonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	**Target**	**Units in Assay**	**Units in Assay MegaKit**
BCL1/JH Tube	MTC of *CCND1* + *IGH* JH	1 x 1500 µL tube	10 x 1500 µL tubes
Specimen Control Size Ladder	Multiple Genes	1 x 1500 µL tube	10 x 1500 µL tubes

Agarose Gel Electrophoresis Detection

The data shown was generated using the master mix indicated. Amplified products were run on a 2% agarose/TBE gel. Lane 1 is data generated testing the recommended 100% clonal control DNA; lane 2 is data generated testing a 10% dilution of the recommended clonal control DNA; lane 3 is data generated testing a 1% dilution of the recommended clonal control DNA; lane 4 is data generated testing a 0.1% dilution of the recommended clonal control DNA; and, lane 5 is data generated testing IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). This control is used as a negative control for many of our tests. A standard 100 base pair DNA size ladder was run in the lanes flanking the test samples.



Ordering Information

Catalog #	Products	Quantity
1-308-0010	*BCL1*/JH Translocation Assay - Gel Detection	33 reactions
1-308-0020	*BCL1*/JH Translocation Assay MegaKit - Gel Detection 330 reactions	330 reactions

BCL2/JH t(14;18) Translocation Assay

Assay Use

The *BCL2*/JH t(14;18) Translocation Assay is useful for studies involving:

- Monitoring and evaluation of follicular lymphomas and other B-cell lymphomas
- Distinguishing lymphoma from benign lymphoid hyperplasia
- Distinguishing follicular lymphoma from other B-cell lymphomas that may have a similar appearance
- Monitoring and evaluation of disease recurrence
- Detection and assessment of residual disease
- Evaluation of new research and methods in malignancy studies

Summary and Explanation of the Test

Five master mixes are included in this assay kit. Two master mixes target *BCL2* major break point (MBR) translocations and two target *BCL2* minor cluster region (mcr) translocations. An Amplification Control Master Mix is also included to ensure the quality and quantity of sample DNA. Positive and negative controls are also included. This assay can be run either in a standard or nested assay format.

Using the standard method, the limit of detection is one cell in one hundred normal cells. The nested method has a limit of detection of one t(14;18) positive cell in a background of ten thousand normal cells. PCR products can be analyzed by standard gel electrophoresis with ethidium bromide staining. A *BCL2* translocation is indicated if just one of the 2nd round master mixes (mixes ending in b) generates product(s) within the valid size range.

Background

The *IGH-BCL2* t(14;18)(q32;q21) translocation is found in 80-90% of follicular lymphomas and in 30% of diffuse large cell lymphomas[1,2]. The translocation is rarely present in other lymphoproliferative diseases[2]. The t(14;18) brings about juxtaposition of *BCL2* with the Ig heavy chain joining segment. This leads to a marked increase in expression of *BCL2* driven by the Ig heavy chain gene enhancer[1]. The *BCL2* protein inhibits programmed cell death (apoptosis) leading to cell accumulation[2]. The majority of breakpoints on 18q21-22 occur within the major breakpoint region (MBR) in the 3′ untranslated region of exon 3 (60-70% of the cases), and the minor cluster region (mcr) located 3′ to *BCL2* exon 3 (20-25% of the cases)[1]. Some breakpoints occur at distant loci and will not be identified by this particular test[2]. Therefore, a negative result does not completely exclude the presence of a *IGH-BCL2* gene rearrangement in the sample. In comparison, Southern blot analysis requires 1-2 weeks, is significantly less sensitive, and has more restrictive Specimen requirements.

Specimen Requirements

1. 5 mL of peripheral blood, bone marrow biopsy, or bone marrow aspirate anti-coagulated with heparin or EDTA; or,
2. Minimum 5 mm cube of tissue; or,
3. 2 µg of genomic DNA; or,
4. Formalin-fixed, paraffin-embedded (FFPE) tissue or slides.

Reference

1. MS Lee et al., *Science* 237: 175-178 (1987).
2. M Crescenzi et al., *Proc. Natl. Acad. Sci. USA* 85: 4869-4873 (1988).

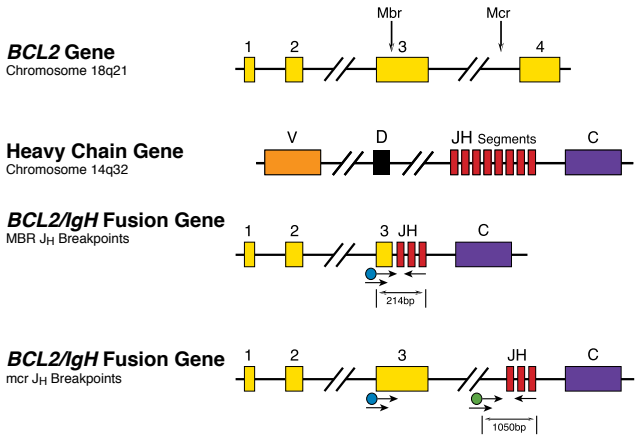


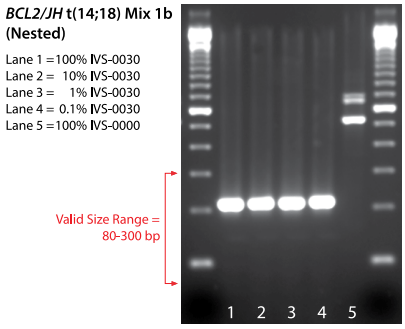
Figure Legend: Simplified view of the genomic organization of the *BCL2* and *IGH* genes on chromosomes 18 and 14, respectively. Yellow boxes represent the exon regions of the *BCL2* gene. Exons of the immunoglobulin heavy chain gene are represented in other colors. The solid black lines represents intron regions, which have been left incompletely spliced to assist in demarcation of the exon segments. MBR and mcr type t(14;18) translocations are shown in the lower portions of the figure with the relative positions of primers and the size of the amplicons generated from the positive control DNAs indicated.

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0030 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0031 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0000 Polyclonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	**Target**	**Units in Assay**	**Units in Assay MegaKit**
*BCL2/*JH t(14;18) (MBR) Mix 1b	Inside *BCL2* MBR	1 x 1500 µL tube	10 x 1500 µL tubes
*BCL2/*JH t(14;18) (mcr) Mix 2b	Inside *BCL2* mcr	1 x 1500 µL tube	10 x 1500 µL tubes
*BCL2/*JH t(14;18) (MBR) Mix 1a	Outside *BCL2* MBR	1 x 1500 µL tube	10 x 1500 µL tubes
*BCL2/*JH t(14;18) (mcr) Mix 2a	Outside *BCL2* mcr	1 x 1500 µL tube	10 x 1500 µL tubes
Amplification Control	*HLA-DQa*	1 x 1500 µL tube	10 x 1500 µL tubes

Agarose Gel Electrophoresis Detection

The data shown was generated using the master mix indicated. Amplified products were run on a 2% agarose/TBE gel. Lane 1 is data generated testing the recommended 100% clonal control DNA; lane 2 is data generated testing a 10% dilution of the recommended clonal control DNA; lane 3 is data generated testing a 1% dilution of the recommended clonal control DNA; lane 4 is data generated testing a 0.1% dilution of the recommended clonal control DNA; and, lane 5 is data generated testing IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). The IVS-0000 control is used as a negative control for many of our tests. A standard 100 base pair DNA size ladder was run in the lanes flanking the test samples.



Ordering Information

Catalog #	Products	Quantity
1-309-0010	*BCL2/*JH t(14;18) Translocation Assay - Gel Detection	33 reactions
1-309-0030	*BCL2/*JH t(14;18) Translocation Assay MegaKit - Gel Detection	330 reactions

BCL2/JH Translocation Assay

Assay Use

The *BCL2*/JH Translocation Assay is useful for studies involving:

- Monitoring and evaluation of follicular lymphomas and other B-cell lymphomas
- Distinguishing lymphoma from benign lymphoid hyperplasia
- Distinguishing follicular lymphoma from other B-cell lymphomas that may have a similar appearance
- Monitoring and evaluation of disease recurrence
- Detection and assessment of residual disease
- Evaluation of new research and methods in malignancy studies

Summary and Explanation of the Test

Four master mixes are included in this assay. Three are used to identify translocations in the major breakpoint region (MBR) and minor cluster region (mcr) of *BCL2*. The Specimen Control Size Ladder master mix targets multiple genes and generates a series of amplicons of approximately 100, 200, 300, 400, and 600 base pairs to ensure that the quality and quantity of input DNA is adequate to yield a valid result. This assay includes negative control DNA and positive control DNAs for both the MBR and mcr. PCR products can be analyzed using standard gel electrophoresis with ethidium bromide staining. A *BCL2* translocation is indicated if any one of the master mixes generates product(s) within the valid size range.

Background

The *BCL2* t(14;18)(q32;q21) translocation is found in 80-90% of follicular lymphomas and 30% of diffuse large cell lymphomas. The translocation is rarely present in other lymphoproliferative diseases.

The t(14;18) brings about juxtaposition of *BCL2* with the Ig heavy chain joining segment. This leads to a marked increase in expression of *BCL2* driven by the Ig heavy chain gene enhancer. The bcl-2 protein inhibits programmed cell death (apoptosis) leading to cell accumulation. The majority of breakpoints on 18q21-22 occur within the major breakpoint region (MBR) in the 3' untranslated region of exon 3 (60- 70% of the cases), and the minor cluster (mcr) region located 3' to *BCL2* exon 3 (20-25% of the cases). Some breakpoints occur at distant loci and will not be identified by this particular test. Therefore, a negative result does not completely exclude the presence of a *BCL2*/*IGH* gene rearrangement in the sample[1]. In comparison, Southern blot analysis requires 1-2 weeks, is significantly less sensitive, and has more restrictive Specimen requirements. The performance characteristics of this assay have been independently determined by the EuroClonality/BIOMED-2 Group. This consortium of 47 molecular diagnostic laboratories tested hundreds of clinical samples, taking into account their lymphoproliferative status as defined by the WHO classifications[2].

Specimen Requirements

1. 5 mL of peripheral blood, bone marrow biopsy, or bone marrow aspirate anti-coagulated with heparin or EDTA; or,
2. Minimum 5 mm cube of tissue; or,
3. 2 µg of genomic DNA; or,
4. Formalin-fixed, paraffin-embedded (FFPE) tissue or slides.

Reference

1. PAS Evans et al., *Leukemia* 17: 2298-2301 (2003).
2. JJM van Dongen et al., *Leukemia* 17: 2257-2317 (2003).

EuroClonality **This assay is based on the EuroClonality/BIOMED-2 Concerted Action BMH4-CT98-3936.**

Figure Legend: Schematic diagram of the *IGH-BCL2* t(14;18) translocation showing the *BCL2* gene on the left and the Ig heavy chain (*IGH*) gene on the right. Shown are the relative positions and orientations for the major breakpoint region (MBR) primers, the minor cluster region (mcr) primers, and the JH primer, which are included in the 3 *BCL2*/JH master mix tubes.

t(14;18) tube A: 2 *BCL2* MBR primers + 1 JH primer

t(14;18) tube B: 4 *BCL2* 3'MBR primers + 1 JH primer

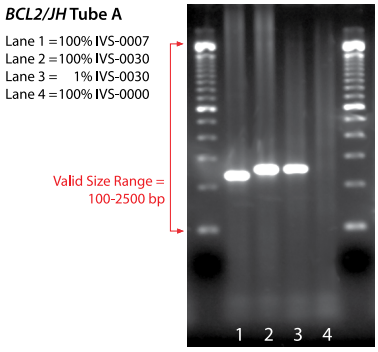
t(14;18) tube C: 3 *BCL2* mcr primers + 1 JH primer

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0030 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-P002 Clonal Control DNA	1600 pg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0031 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0000 Polyclonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	**Target**	**Units in Assay**	**Units in Assay MegaKit**
BCL2/JH Tube A	*BCL2* MBR + *IGH* JH	1 x 1500 µL tube	10 x 1500 µL tubes
BCL2/JH Tube B	*BCL2* 3' MBR + *IGH* JH	1 x 1500 µL tube	10 x 1500 µL tubes
BCL2/JH Tube C	*BCL2* mcr + *IGH* JH	1 x 1500 µL tube	10 x 1500 µL tubes
Specimen Control Size Ladder	Multiple Genes	1 x 1500 µL tube	10 x 1500 µL tubes

Agarose Gel Electrophoresis Detection

The data shown was generated using the master mix indicated. Amplified products were run on a 2% agarose/TBE gel. Lane 1 is data generated testing an alternative 100% clonal control DNA; lane 2 is data generated testing the recommended 100% clonal control DNA; lane 3 is data generated testing a 1% dilution of the recommended clonal control DNA; and, lane 4 is data generated testing IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). The IVS-0000 control is used as a negative control for many of our tests. A standard 100 base pair DNA size ladder was run in the lanes flanking the test samples.



Ordering Information

Catalog #	Products	Quantity
1-309-0020	*BCL2*/JH Translocation Assay - Gel Detection	33 reactions
1-309-0040	*BCL2*/JH Translocation Assay MegaKit - Gel Detection	330 reactions

BCR/ABL t(9;22) Translocation Assays

Assay Use

BCR/ABL t(9;22) Translocation Assays are useful for studies involving:

- Identification of chronic myeloid leukemia (CML), acute lymphoblastic leukemia (ALL)
- Monitoring and evaluation of disease recurrence
- Detection and assessment of residual disease
- Evaluation of new research and methods in malignancy studies

Summary and Explanation of the Test

The master mixes are included in these assay kits used to amplify complementary DNA (cDNA) produced from specimen(s), and positive and negative RNA controls (included). Primers target an internal control transcript (*Abl*) and p190-, p210-, and p230-type transcripts expressed from *BCR-ABL1* translocations. The limit of detection of this assay is approximately one *BCR-ABL1* positive cell in a background of one million normal cells. Amplicon products can be analyzed by capillary electrophoresis or standard gel electrophoresis with ethidium bromide staining. A *BCR-ABL1* translocation is indicated if just one of the 2nd round master mixes (Mix 2b, Mix 2c, Mix 3b, Mix 3c, or Mix 3d) generates product(s) of the valid size. Reagents for RNA extraction and reverse transcription are not included. This assay is compatible with all standard RNA extraction and cDNA synthesis methods. This is a qualitative assay and has not been validated for quantitative use.

Background

The Philadelphia chromosome (Ph1) is a specific chromosomal abnormality that results from reciprocal t(9;22)(q34;q11) chromosome rearrangements that fuse coding regions of the *BCR* gene, located on chromosome 22, with the *ABL* receptor independent tyrosine kinase gene on chromosome 9[1,2]. *BCR-ABL1* t(9;22) translocations are present in approximately 95% of chronic myeloid leukemia (CML) patients, 20-50% of adult acute lymphoblastic leukemia (ALL) patients, and 2-10% of pediatric ALL patients[2]. Although cytogenetic detection of Ph1 is a hallmark of CML, molecular detection of Ph1-positive cells by nested reverse transcriptase PCR is faster and significantly more sensitive than cytogenetics or other methods. Nearly 50% of cytogenetically Ph1-negative CML cases are positive by reverse transcriptase PCR analysis. This makes reverse transcriptase PCR detection of Ph1-positive cells of value in predicting early disease recurrence and progression for CML and ALL patients that are in apparent clinical remission following bone marrow transplantation[3]. Molecular detection provides the opportunity for early intervention and treatment. Thus, molecular testing for chimeric *BCR-ABL1* transcripts is utilized both in diagnostic evaluation and post-therapeutic monitoring of CMLs and ALLs.

Specimen Requirements

1. 5 mL of peripheral blood, bone marrow biopsy, or bone marrow aspirate anticoagulated with heparin or EDTA; or,
2. Archived cells frozen in 10% DMSO + 90% Fetal Bovine Serum (FBS).

Reference

1. R Kurzrock et al., *Ann. Intern. Med.* 138: 819-30 (2003).
2. JV Melo. *Blood* 88: 2375-2384 (1996).
3. JP Radich et al., *Blood* 85: 2632-2638 (1995).

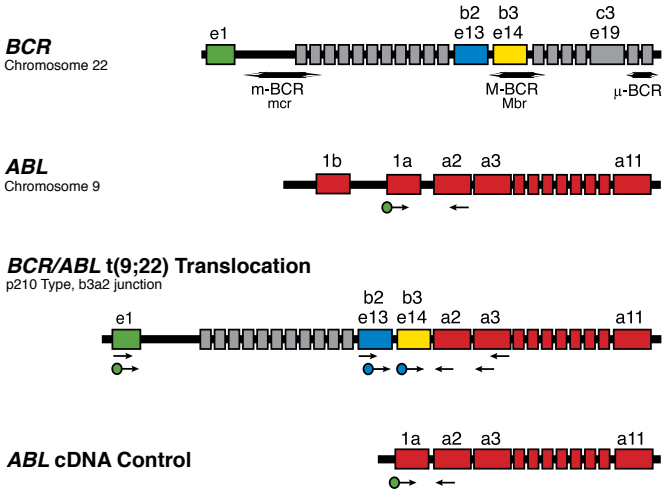


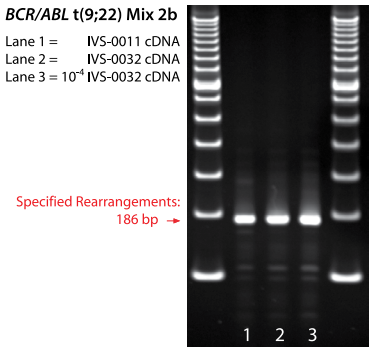
Figure Legend: This figure shows the genomic organization of the *BCR* and *ABL* genes on chromosomes 22 and 9, respectively. Boxes represent exon regions of the *ABL* (red boxes) and *BCR* encoding exons (other colors). The solid black line represents intron regions, which have been left incompletely spliced to assist in demarcation of the exon segments. The location of exon regions targeted by labeled and unlabeled primers are indicated by arrows. A p210-type *BCR-ABL1* translocation (b3a2 junction) is depicted in the lower portion of the figure along with the control *ABL* transcript control.

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0032 Clonal Control RNA	400 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0011 Clonal Control RNA	400 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0035 Clonal Control RNA	400 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	**Target**	**Units in Assay**	**Units in Assay MegaKit**
BCR/ABL t(9;22) Mix 1a	*Abl*	1 x 1500 µL tube	10 x 1500 µL tubes
BCR/ABL t(9;22) Mix 2a	p190	1 x 1500 µL tube	10 x 1500 µL tubes
BCR/ABL t(9;22) Mix 3a	p210+230	1 x 1500 µL tube	10 x 1500 µL tubes
BCR/ABL t(9;22) Mix 1b	*Abl*	1 x 1500 µL tube	10 x 1500 µL tubes
BCR/ABL t(9;22) Mix 2b	p190	1 x 1500 µL tube	10 x 1500 µL tubes
BCR/ABL t(9;22) Mix 2c	p190	1 x 1500 µL tube	10 x 1500 µL tubes
BCR/ABL t(9;22) Mix 3b	p210+230	1 x 1500 µL tube	10 x 1500 µL tubes
BCR/ABL t(9;22) Mix 3c	p210+230	1 x 1500 µL tube	10 x 1500 µL tubes
BCR/ABL t(9;22) Mix 3d	p210+230	1 x 1500 µL tube	10 x 1500 µL tubes

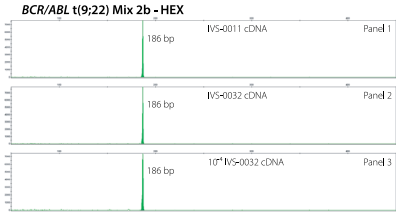
Polyacrylamide Gel Electrophoresis Detection

The data shown was generated using the master mix indicated. Amplified products were run on a 6% polyacrylamide/TBE gel. Lane 1 is data generated testing cDNA synthesized from an alternative 100% clonal control RNA; lane 2 is data generated testing cDNA synthesized from the recommended 100% clonal control RNA; lane 3 is data generated testing cDNA synthesized from a 10^{-4} dilution of the recommended clonal control RNA. The positive RNA was diluted into the negative control RNA, IVS-0035. A standard 100 base pair DNA size ladder was run in the lanes flanking the test samples.



Capillary Electrophoresis Detection (ABI)

The data shown was generated on an ABI 3100 instrument using the master mix indicated. Panel 1 represents data generated testing cDNA synthesized from an alternative 100% control RNA; panel 2 represents data generated testing cDNA synthesized from the recommended 100% clonal control RNA; and, panel 3 represents data generated testing cDNA synthesized from a 10^{-4} dilution of the recommended clonal control RNA. The positive RNA was diluted into the negative control RNA, IVS-0035 (Cat# 4-089-3070).



Ordering Information

Catalog #	Products	Quantity
1-310-0010	*BCR/ABL* t(9;22) Translocation Assay - Gel Detection	33 reactions
1-310-0020	*BCR/ABL* t(9;22) Translocation Assay MegaKit - Gel Detection	330 reactions
1-310-0031	*BCR/ABL* t(9;22) Translocation Assay - ABI Fluorescence Detection	33 reactions
1-310-0041	*BCR/ABL* t(9;22) Translocation Assay MegaKit - ABI Fluorescence Detection	330 reactions

PML/RARa t(15;17) Translocation Assays

Assay Use

PML/RARα t(15;17) Translocation Assays are useful for studies involving:

- Identification of acute promyelocytic leukemia (APL)
- Monitoring and evaluation of disease recurrence
- Detection and assessment of residual disease
- Evaluation of new research and methods in malignancy studies

Summary and Explanation of the Test

Four master mixes are included in these assay kits. Master mixes are used to amplify complementary DNA (cDNA) produced from specimen(s), as well as positive and negative RNA controls (included). Primers target an internal control transcript (*RARA*, formerly known as *RARα*) and the variety of Bcr1, Bcr2, and Bcr3 type transcripts expressed from *PML-RARA* translocations. The limit of detection for this assay is one *PML-RARA* positive cell in a background of one hundred thousand normal cells. Amplicon products can be analyzed by differential fluorescence detection using capillary electrophoresis or standard gel electrophoresis. A *PML-RARA* translocation is indicated if just one of the 2nd round master mixes (Mix 2b or Mix 2c) generates product(s) of the valid size. Reagents for RNA extraction and reverse transcription are not included. This assay is compatible with all standard RNA extraction and cDNA synthesis methods. This is a qualitative assay and has not been validated for quantitative use.

Background

Acute promyelocytic (M3) leukemia (APL) is a distinct form of acute myeloid leukemia (AML) representing approximately 10% of AMLs. These leukemias often express *PML-RARA* transcripts from t(15;17) chromosomal translocations that fuse the *PML* (or *MYL*) gene on chromosome 15 with the retinoic acid receptor a (*RARA*) gene on chromosome 17[1,2,3,4]. Diagnosis of APL is typically based upon identification of promyelocytes with distinctive morphology plus cytogenetic or molecular detection of these t(15;17) translocations[4]. Three *PML/RARA* translocation patterns have been identified: Type A is the short form (S-form); the breakpoint occurs within the breakpoint cluster region 3 (Bcr3). Type B is the long form (L-form) and the breakpoint occurs within Bcr1. There is a third type B variant or variable form (V-form) whose breakpoint occurs within Bcr2. Identification of the *PML-RARA* translocation is important in APL because it is correlated with responsiveness to treatment with all-trans retinoic acid (ATRA). Patients incorrectly diagnosed with APL by clinical and morphologic criteria alone are typically unresponsive to treatment with ATRA. APL patients are also at risk for disseminated intravascular coagulation (DIC), which can become more severe during conventional chemotherapy. As a result, there is clearly a need for a rapid, sensitive, and reliable molecular assay that identifies *PML-RARA* transcripts associated with APL. This Assay uses both non-nested (master mix 1) and nested (master mixes 2a, 2b, and 2c) reverse transcriptase PCR for faster and significantly more sensitive results than cytogenetics or other methods.

Specimen Requirements

1. 5 mL of peripheral blood, bone marrow biopsy, or bone marrow aspirate anticoagulated with heparin or EDTA; or,
2. Archived cells frozen in 10% DMSO + 90% Fetal Bovine Serum (FBS).

Reference

1. H De Thé et al., *Nature* 347: 558-561 (1990).
2. H De Thé et al., *Cell* 66: 675-684 (1991).
3. A Kakizuka et al., *Cell* 66: 663-674 (1991).
4. WH Miller et al., *Proc. Natl. Acad. Sci.* 89: 2694-2698 (1992).

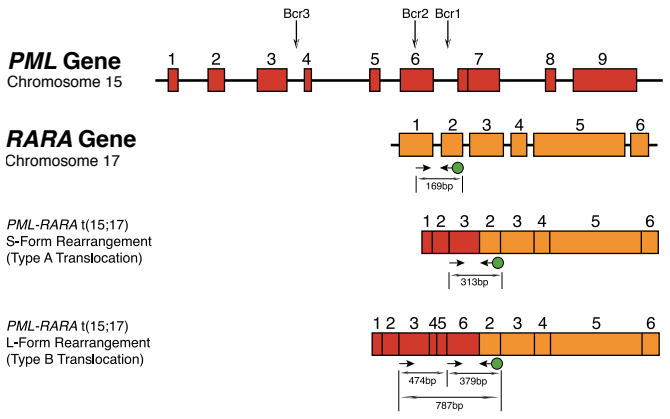


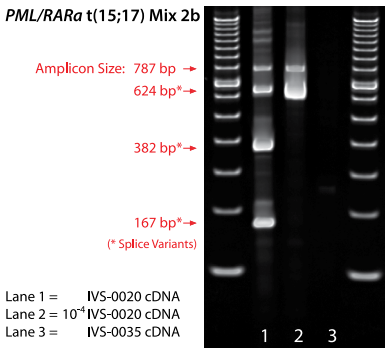
Figure Legend: This figure shows the genomic organization of the PML and RARA genes on chromosomes 15 and 17, respectively. Boxes represent exon regions of the *PML* (red boxes) and *RARA* (orange) encoding exons. The solid black line represents intron regions, which were left incompletely spliced to assist in demarcation of the exon segments. Primers are indicated by arrows, and the size of several of the products are indicated below the translocated gene segments. S-form (Bcr3) and L-form (Bcr1) *PML-RARA* translocations are depicted in the lower portion of the figure.

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0020 Clonal Control RNA	400 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0035 Clonal Control RNA	400 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	**Target**	**Units in Assay**	**Units in Assay MegaKit**
PML/RARα t(15;17) Mix 1	*RARA*	1 x 1500 µL tube	10 x 1500 µL tubes
PML/RARα t(15;17) Mix 2a	*PML-RARA*	1 x 1500 µL tube	10 x 1500 µL tubes
PML/RARα t(15;17) Mix 2b	S- and L-Forms	1 x 1500 µL tube	10 x 1500 µL tubes
PML/RARα t(15;17) Mix 2c	L-Form	1 x 1500 µL tube	10 x 1500 µL tubes

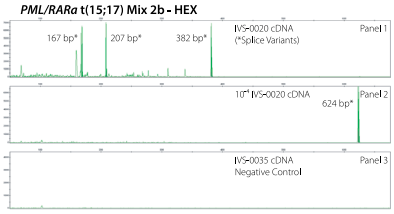
Polyacrylamide Gel Electrophoresis Detection

The data shown were generated using the master mix indicated. Amplified products were run on a 6% polyacrylamide/TBE gel. Lane 1 is data generated testing cDNA synthesized from the recommended 100% clonal control RNA; lane 2 is data generated testing cDNA synthesized from a 10^{-4} dilution of the recommended clonal control RNA; and, lane 3 is data generated testing cDNA synthesized from the negative control the negative control IVS-0035 Clonal Control RNA (Cat# 4-089-3070). A standard 100 base pair DNA size ladder was run in the lanes flanking the test samples.



Capillary Electrophoresis Detection (ABI)

The data shown were generated on an ABI 3100 instrument using the master mix indicated. Panel 1 represents data generated testing cDNA synthesized from the recommended 100% clonal control RNA; panel 2 represents data generated testing cDNA synthesized from a 10^{-4} dilution of the recommended clonal control RNA; and, panel 3 represents data generated testing cDNA synthesized from the negative control RNA. The positive RNA was diluted into the negative control RNA, IVS-0035 (Cat# 4-089-3070).



Ordering Information

Catalog #	Products	Quantity
1-311-0010	*PML/RARα* t(15;17) Translocation Assay - Gel Detection	33 reactions
1-311-0020	*PML/RARα* t(15;17) Translocation Assay MegaKit - Gel Detection	330 reactions
1-311-0011	*PML/RARα* t(15;17) Translocation Assay - ABI Fluorescence Detection	33 reactions
1-311-0021	*PML/RARα* t(15;17) Translocation Assay MegaKit - ABI Fluorescence Detection	330 reactions

IGH Somatic Hypermutation Assays v2.0

Assay Use

IGH Somatic Hypermutation Assays are useful for studies involving:

- Identifying clonal rearrangements of the immunoglobulin heavy chain (*IGH*) gene
- Assessing the extent of somatic hypermutation (SHM) in the variable (VH) gene sequence (*IGHV*) in patients with chronic lymphocytic leukemia (CLL) and small lymphocytic lymphoma (SLL)
- Evaluating new research and methods in malignancy studies

Summary and Explanation of the Test

These assays amplify either genomic DNA or complementary DNA (cDNA) that lies between the upstream leader (VHL) or framework 1 (FR1) regions and the downstream joining (JH) region of the *IGH* gene. The assays employ two different master mixes: Hypermutation Mix 1 and Hypermutation Mix 2. The Hypermutation Mix 1 targets sequences between the leader (VHL) and joining (JH) regions. Therefore the amplicon product(s) span the entire variable (VH) region, which contains all framework (FR) and complementarity-determining regions (CDR). The Hypermutation Mix 2 targets sequences between the framework 1 (FR1) and joining (JH) regions. The resulting amplicons include a portion of the FR1 region to the downstream JH region. The primers that target the VHL and FR1 regions have been redesigned to include a universal sequencing tag at the 5'-end. This new design allows for bi-directional sequencing of clonal PCR products with just one sequencing-tag specific forward primer and one JH reverse primer, thus ensuring a more reliable and complete coverage of clonal products. Current ERIC (European Research Initiative on CLL) guidelines recommend bi-directional sequencing when determining the *IGH* SHM status. Positive and negative DNA, positive RNA, as well as an amplification control are included in the assay. Clonality is indicated if any one of the master mixes generates clonal products.

Background

Immunoglobulin variable heavy chain gene hypermutation status provides important prognostic information for patients with chronic lymphocytic leukemia (CLL) and small lymphocytic lymphoma (SLL). The presence of *IGH* somatic hypermutation (SHM) is defined as greater or equal to 2% difference from the germline variable (VH) gene sequence, whereas less than 2% difference is considered evidence of no somatic hypermutation. This has clinical relevance, as there is a clear distinction in the median survival of patients with and without somatic hypermutation. Hypermutation of the *IGHV* gene is strongly predictive of a good prognosis while lack of mutation predicts a poor prognosis. This assay aids in identification, sequencing, and analysis of somatic hypermutation status of clonal products. PCR products can be analyzed by gel electrophoresis detection or by differential fluorescence detection using capillary electrophoresis followed by gel electrophoresis detection. PCR products are sequenced bidirectionally either directly, after gel extraction, or after cloning into a bacterial vector. The resulting sequence is then compared to *IGH* germline sequences to determine mutational status.

Specimen Requirements

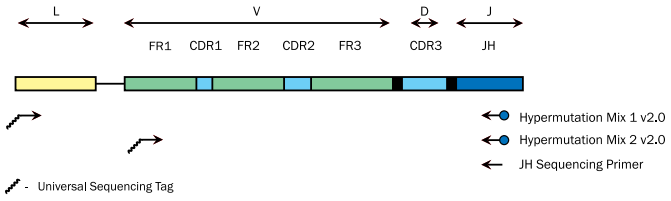
These assays test genomic DNA or cDNA from the following sources:

1. 5 mL of peripheral blood, bone marrow biopsy, or bone marrow aspirate anti-coagulated with heparin or EDTA; or,
2. Minimum 5 mm cube of tissue; or,
3. 2 µg of genomic DNA; or,
4. 5 µg of total RNA or mRNA; or,
5. 1 µg of cDNA; or,
6. Formalin-fixed, paraffin-embedded (FFPE) tissue or slides.

Reference

1. P Ghia et al., *Leukemia* 21: 1-3 (2007).
2. P Ghia et al., *Blood* 105: 1678-1685 (2005).
3. F Davi et al., *Leukemia* 22: 212-214 (2008).

Figure Legend: Simple representation of the organization of a rearranged immunoglobulin heavy chain gene on chromosome 14. Black arrows represent the relative positions of primers that target the conserved Leader (L) and Framework 1 (FR1) regions, and the downstream consensus JH gene segments.

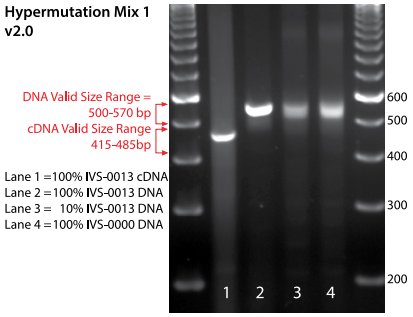


Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0013 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0013 Clonal Control RNA	400 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0000 Polyclonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	**Target**	**Units in Assay**	**Units in Assay MegaKit**
Hypermutation Mix 1 v2.0	Leader + JH	1 x 1500 µL tube	10 x 1500 µL tubes
Hypermutation Mix 2 v2.0	Framework 1 + JH	1 x 1500 µL tube	10 x 1500 µL tubes
Specimen Control Size Ladder	Multiple Genes	1 x 1500 µL tube	10 x 1500 µL tubes
Primers	**Target**	**Units in Assay**	**Units in Assay MegaKit**
Primer - Hypermutation	Leader + Framework 1	1 x 10 µL tube at 100 µM	5 x 10 µL tube
IGH JH Primer	JH	1 x 10 µL tube at 100 µM	5 x 10 µL tube

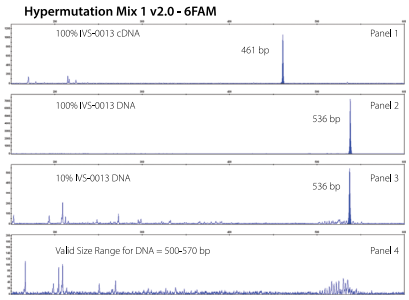
Polyacrylamide Gel Electrophoresis Detection

The data shown was generated using the master mix indicated. Amplified products were run on a 2% agarose/TBE gel. Lane 1 is data generated testing cDNA synthesized from the recommended 100% clonal control RNA; lane 2 is data generated testing the recommended 100% clonal control DNA; lane 3 is data generated testing a 10% dilution of the recommended clonal control DNA; and, lane 4 is data generated testing the IVS-0000 Polyclonal Control DNA (Cat# 4-092- 0010). Lanes flanking the samples were loaded with a 100 base pair DNA size standard.



Capillary Electrophoresis Detection (ABI)

The data shown was generated on an ABI 3130xl instrument using the master mix indicated. Panel 1 displays data generated testing cDNA synthesized from the recommended 100% clonal control RNA; panel 2 displays data generated testing the recommended 100% clonal control DNA; panel 3 displays data generated testing the recommended 10% clonal control DNA; and panel 4 displays data generated testing IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010).



Ordering Information

Catalog #	Products	Quantity
5-101-0030	*IGH* Somatic Hypermutation Assay v2.0 - Gel Detection	33 reactions
5-101-0040	*IGH* Somatic Hypermutation Assay v2.0 MegaKit - Gel Detection	330 reactions
5-101-0031	*IGH* Somatic Hypermutation Assay v2.0 - ABI Fluorescence Detection	33 reactions
5-101-0041	*IGH* Somatic Hypermutation Assay v2.0 MegaKit - ABI Fluorescence Detection	330 reactions

FLT3 Mutation Assays

These products are not currently available for sale or for use in the United States

Assay Use

FLT3 Mutation Assays are useful for the study of:

- Identifying *FLT3* mutations in patients with AML
- Discriminating between high and low risk patients. *FLT3* mutations portend a worse prognosis for patients with AML. Therefore patients testing positive for *FLT3* mutations may benefit from a more aggressive treatment regimen

Summary and Explanation of the Test

Acute myeloid leukemia (AML) in general has a poor prognosis. Recent studies have described mutation of the *FLT3* (fms-related tyrosine kinase 3) receptor to be the most important prognostic factor in AML, with *FLT3* mutants having a worse outcome and response to standard chemotherapeutic interventions. Accordingly, identification of an *FLT3* mutation in AML may indicate a need to reassess and modify standard treatment options.

All types of AML can have activating mutations in the *FLT3* gene. Mutation of the *FLT3* receptor, either by internal tandem duplication (ITD) of the juxtamembrane domain or by point mutation of the aspartic acid residue (D835) or isoleucine (I836) in the activation loop of the kinase domain, causes constitutive activation of the *FLT3* receptor.

This test kit includes 3 master mixes. The ITD and D835 master mixes target the juxtamembrane and kinase domain regions (respectively). The third master mix, the Specimen Control Size Ladder, targets multiple genes and generates a series of amplicons of 100, 200, 300, 400, and 600 base pairs to ensure that the quality and quantity of input DNA is adequate to yield a valid result.

PCR products can be analyzed by capillary electrophoresis with use of ABI instruments or standard agarose TBE gel electrophoresis with ethidium bromide staining.

Background

Mutations in the fms related tyrosine kinase 3 (*FLT3*) gene are the most common mutations found in acute myeloid leukemia (AML), occurring in approximately 30% of patients at the time of diagnosis, and are characterized by an aggressive phenotype with a high prevalence of relapse[1,2,3].

The most prevalent and clinically significant type of *FLT3* mutation is an internal tandem duplication (ITD) in the juxtamembrane domain[4]. Many clinical studies have found that *FLT3* ITD mutations are associated with higher concentrations of leukemic cells in both blood and bone marrow, increased incidence of relapse, and decreased overall survival.

The second most common mutation type in the *FLT3* gene is a tyrosine kinase domain (TKD) point mutation in aspartate (D835) or isoleucine (I836). TKD mutations result in constitutive autophosphorylation and activation of *FLT3*[5,6]. TKD mutations have been linked to poor overall survival, but to a lesser extent as compared to ITD mutations.

Specimen Requirements

1. 5 cc of peripheral blood, bone marrow biopsy, or bone marrow aspirate anti-coagulated with heparin or EDTA; or,
2. Minimum 5 mm cube of tissue; or,
3. 2 µg of genomic DNA; or,
4. Formalin-fixed, paraffin-embedded tissue or slides.

Reference

1. Acute Myeloid Leukemia, Clinical Practice Guidelines in Oncology, National Comprehensive Cancer Network (v.2.2014).
2. Lowenberg, B. et al. "Acute myeloid leukemia." N Engl J Med 341(14):1051-62 (1999).
3. Thiede, C. et al. "Analysis of *FLT3*-activating mutations in 979 patients with acute myelogenous leukemia: association with FAB and identification of subgroups with poor prognosis." Blood 99(12): 4326-35 (2002).
4. Nakao, M. et al. "Internal tandem duplication of the *FLT3* gene found in acute myeloid leukemia." Leukemia 10(12):1911-18 (1996).
5. Yamamoto, Y et al. Activating mutation of D835 within the activation loop of *FLT3* in human hematologic malignancies. Blood, 97(8):2434-9 (2001).
6. Gilliland, DG et al. The roles of *FLT3* in hematopoiesis and leukemia. Blood 100(5):1532-154 (2002).

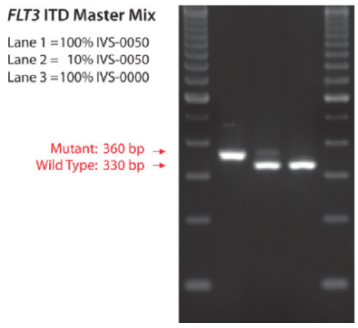
Reagents

Controls	Concentration	Units in 33 Reaction Assay	Units in 330 Reaction MegaKit
IVS-0017 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-P001 Clonal Control DNA	200 pg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0000 Polyclonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	**Target**	**Units in 33 Reaction Assay**	**Units in 330 Reaction MegaKit**
FLT3 ITD Master Mix	*FLT3* ITD	1 x 1500 µL tube	10 x 1500 µL tubes
FLT3 D835 Master Mix	*FLT3* TKD	1 x 1500 µL tube	10 x 1500 µL tubes
Specimen Control Size Ladder	Multiple Genes	1 x 1500 µL tube	10 x 1500 µL tubes

Gel Electrophoresis Detection

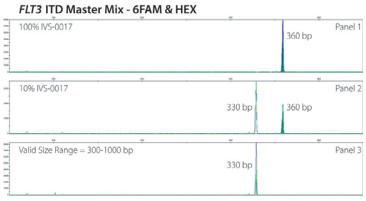
Data was generated using the *FLT3* ITD Master Mix and amplified products were run on a 2% agarose TBE gel. Lane 1 is a *FLT3* ITD control*; lane 2 is a 10% dilution of a *FLT3* ITD control; and lane 3 is IVS-0000, which is representative of a WT product. A standard 100 bp DNA size ladder was run in the lanes flanking the test samples.

*IVS-0050 performs comparable to IVS-0017 clonal control DNA, which is included in the kit as the positive control.



Capillary Electrophoresis Detection (ABI)

Data was generated using the *FLT3* ITD Master Mix and amplified products were run on an ABI 3100 instrument. Panel 1 is the recommended *FLT3* ITD positive control; panel 2 is data generated testing a 10% dilution of the positive control; and Panel 3 is IVS-0000, which is representative of a WT product.



Ordering Information

Catalog #	Products	Quantity
1-412-0010	*FLT3* Mutation Assay - Gel Detection	33 reactions
1-412-0020	*FLT3* Mutation Assay MegaKit - Gel Detection	330 reactions
1-412-0031	*FLT3* Mutation Assay - ABI Fluorescence Detection	33 reactions
1-412-0041	*FLT3* Mutation Assay MegaKit - ABI Fluorescence Detection	330 reactions

These products are intended for Research Use Only. Not for use in diagnostic procedures.

FLT3 internal tandem duplication testing is covered by United States Patent number 6,846,630 and 8,178,292, licensed exclusively to Invivoscribe Technologies

Use of this product may require nucleic acid amplification methods such as Polymerase Chain Reaction (PCR). Any necessary license to practice amplification methods or to use amplification enzymes or equipment covered by third party patents is the responsibility of the user and no such license is granted by Invivoscribe Technologies, Inc., expressly or by implication.

For additional patent information, contact our legal department by email at legal@invivoscribe.com, or by telephone in the United States: 1 (858) 224-6600.

Warranty and Liability

Invivoscribe Technologies, Inc. (Invivoscribe®) is committed to providing the highest quality products. Invivoscribe® warrants that the products meet or exceed the performance standards described in the Instructions For Use, as to products with such an insert. If a product is covered by product specifications and does not perform as specified, our policy is to replace the product or credit the full purchase price. No other warranties of any kind, expressed or implied, are provided by Invivoscribe®. Invivoscribe® liability shall not exceed the purchase price of the product. Invivoscribe® shall have no liability for direct, indirect, consequential, or incidental damages arising from the use, results of use, or inability to use its products; product efficacy under purchaser controlled conditions in purchaser's laboratory must be established and continually monitored through purchaser defined and controlled processes including but not limited to testing of positive, negative, and blank controls every time a sample is tested. Ordering, acceptance, and use of product constitutes purchaser acceptance of sole responsibility for assuring product efficacy and purchaser agreement to the limitation of liability set forth in this paragraph.

NOTICE: The products in the section that follows are *in vitro* diagnostic products and are not available for sale or use within North America. Many of these products are covered by one or more of the following: European Patent Number 1549764, European Patent Number 2418287, European Patent Number 2460889, Japanese Patent Number 4708029, United States Patents 6846630, 8178292 and 8859748 and related pending and future applications. All of these patents and applications are licensed exclusively to Invivoscribe®. Additional patents licensed to Invivoscribe covering some of these products apply elsewhere.

These products require nucleic acid amplification methods such as Polymerase Chain Reaction (PCR). No license under these patents to use amplification processes or enzymes is conveyed expressly or by implication to the purchaser by the purchase of these products.

Analyte Specific Reagents

Invivoscribe Analyte Specific Reagents (ASRs) target B- and T-cell antigen receptor loci, as well as *IGH-BCL2, BCR-ABL1,* and *PML-RARA* chromosome translocations.

The ASRs are available as a single tube containing a volume of 1500 µL. To ensure the highest quality and reliability of reagents ASRs are manufactured under cGMP and ISO 13485 standards. The analytical and performance characteristics of the ASRs have not been established.

Per the current US FDA regulations, ASRs may only be sold to *in vitro* diagnostic manufacturers, CLIA accredited high complexity laboratories, VHA regulated clinical laboratories, and laboratories not intending to use ASRs as a part of a diagnostic test.

ASRs are not available for sale or use outside of the USA.

FLT3 (FMS-like Tyrosine Kinase 3)

Description	Catalog #
FLT3 ITD Master Mix - Unlabeled	Please contact Invivoscribe
FLT3 TKD Master Mix - Unlabeled	Please contact Invivoscribe

IGH (Immunoglobulin Heavy Chain Gene Locus)

Description	Catalog #
IGH Framework 1 - 6FAM	A-101-0061
IGH Framework 2 - Unlabeled	A-101-0070
IGH Framework 2 - 6FAM	A-101-0091
IGH Framework 3 - Unlabeled	A-101-0080
IGH Framework 3 - HEX	A-101-0081
IGH FR1 - Unlabeled	A-101-0010
IGH FR1 - 6FAM	A-101-0011
IGH FR2 - Unlabeled	A-101-0020
IGH FR2 - 6FAM	A-101-0101
IGH FR3 - Unlabeled	A-101-0030
IGH FR3 - HEX	A-101-0031
IGH DH1 - 6 - HEX	A-101-0041
IGH DH7 - 6FAM	A-101-0051

IGK (Immunoglobulin Kappa Light Chain Gene Locus)

Description	Catalog #
IGK V - J - Unlabeled	A-102-0010
IGK V - J - 6FAM	A-102-0011
IGK V - Kde - Unlabeled	A-102-0020
IGK V - Kde - 6FAM	A-102-0021

IGL (Immunoglobulin Lambda Light Chain Gene Locus)

Description	Catalog #
IGL V - J - 6FAM	A-103-0011

TRB (T-Cell Receptor Beta Chain Gene Locus)

Description	Catalog #
TCRB V - J1 + 2 - Unlabeled	A-205-0010
TCRB V - J1 + 2 - 6FAM & HEX	A-205-0011
TCRB V - J2 - Unlabeled	A-205-0020
TCRB V - J2 - 6FAM	A-205-0021
TCRB D - J1 + 2 - Unlabeled	A-205-0030
TCRB D - J1 + 2 - 6FAM & HEX	A-205-0031

TRD (T-Cell Receptor Delta Chain Gene Locus)

Description	Catalog #
TCRD V - D - J - 6FAM & HEX	A-206-0011

TRG (T-Cell Receptor Gamma Chain Gene Locus)

Description	Catalog #
TCRG V(2-5,8-11)J 1 + 2+P - 6FAM	A-207-0091
TCRG V(1-8,9)J - 6FAM	A-207-0071
TCRG V(1-8)J - HEX	A-207-0021
TCRG V(1-8,10)J - Unlabeled	A-207-0030
TCRG V(1-8,10)J - 6FAM & HEX	A-207-0031
TCRG V(9,11)J - Unlabeled	A-207-0040
TCRG V(9,11)J - 6FAM & HEX	A-207-0041

IGH-BCL2 t(14;18)

Description	Catalog #
BCL2/JH Mbr - Unlabeled	A-309-0050
BCL2/JH 3'Mbr - Unlabeled	A-309-0060
BCL2/JH mcr - Unlabeled	A-309-0070

BCR-ABL1 t(9;22)

Description	Catalog #
BCR/ABL e1.1-a3.1 - Unlabeled	A-310-0020
BCR/ABL b2.1-a3.1 - Unlabeled	A-310-0030
BCR/ABL, e1.2-a2.2 - HEX	A-310-0051
BCR/ABL, e1.2-a3.2 - HEX	A-310-0061
BCR/ABL, b2.2-a2.2 - 6FAM	A-310-0071
BCR/ABL, b2.2-a3.2 - 6FAM	A-310-0081
BCR/ABL, b3.2-a3.2 - 6FAM	A-310-0101

PML-RARA t(15;17)

Description	Catalog #
PML/RARa, MYL2-RARa - Unlabeled	A-311-0020
PML/RARa, MYL1-RARa - Unlabeled	A-311-0030
PML/RARa, PML3-RARa - Unlabeled	A-311-0040

Warranty and Liability

Invivoscribe Technologies, Inc. (Invivoscribe®) is committed to providing the highest quality products. Invivoscribe® warrants that the products meet or exceed the performance standards described in the Instructions For Use, as to products with such an insert. If a product is covered by product specifications and does not perform as specified, our policy is to replace the product or credit the full purchase price. No other warranties of any kind, expressed or implied, are provided by Invivoscribe®. Invivoscribe® liability shall not exceed the purchase price of the product. Invivoscribe shall have no liability for direct, indirect, consequential or incidental damages arising from the use, results of use, or inability to use its products; product efficacy under purchaser controlled conditions in purchaser's laboratory must be established and continually monitored through purchaser defined and controlled processes including but not limited to testing of internally validated positive, negative, and blank controls every time a sample is tested. Ordering, acceptance and use of product constitutes purchaser acceptance of sole responsibility for assuring product efficacy and purchaser agreement to the limitation of liability set forth in this paragraph.

Notice to Purchaser - EagleTaq DNA Polymerase ONLY
This product is for sale and use in the European Economic Area only. It is not to be resold or transferred to another party. Use of this product is covered by US Patent No. 6,127,155 and corresponding patent claims outside the US. This purchaser of this product may use this amount of product only for the purchaser's own internal research. No right under any other patent claim and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Human and veterinary diagnostic uses under Roche patent claims require a separate license from Roche. All uses other than internal research and human and veterinary diagnostic uses under Roche patent claims require a separate license from Thermo Fisher Scientific. By using this product, you acknowledge your agreement to the above. Further information on purchasing licenses from Roche may be obtained by contacting the Licensing Department of Roche Molecular Systems, Inc., 4300 Hacienda Drive, Pleasanton, California 94588, USA or Roche Diagnostics GmbH, Sandhofer Strasse 116, 68305 Mannheim, Germany. Further information on purchasing licenses from Thermo Fisher Scientific may be obtained by contacting the Licensing Department of Thermo Fisher Scientific, 5791 Van Allen Way, Carlsbad, California 92008, USA.

Controls, Reagents, and Enzymes

Invivoscribe offers an extensive range of General Purpose Reagents (GPRs) and Research Use Only (RUO) nucleic acid controls.

Controls are available in several different formats: plasmid DNA, DNA extracted from tissue or cell lines, or RNA extracted from cell lines. These controls can be purchased in various dilutions or as complete dilution sets and panels for several purposes, such as to help with assay validation, sensitivity or proficiency testing, or troubleshooting.

The following pages will provide an overview of available controls, along with a number of tables and reference guides, to help you decide which Invivoscribe control(s) will be suitable for your application.

DNA Controls

Every laboratory needs suitable controls (positive and negative) for sensitivity and proficiency testing, as well as for troubleshooting. Since patient samples cannot serve as true controls (due to a lack of characterization and inter-sample variability), Invivoscribe offers a multitude of high quality, reliable DNA controls manufactured under cGMP conditions.

These controls can be used for all assays targeting B- and T-cell antigen receptor loci, as well as *IGH-BCL2, BCR-ABL1,* and *PML-RARa* chromosome translocations.

Quick Reference for DNA Controls

Our high-quality DNA controls are supplied in aliquots of 100 µL per tube. Each control is adjusted to a final concentration of 200 µg/mL in 1/10 TE buffer (1 mM Tris-HCl (pH 8.0), 0.1 mM EDTA). The reference tables below list Clonal Control DNA by target and by the name assigned to each clonal control DNA.

Positive for	Immunoglobulin Rearrangements			Mutations	Translocations		T-Cell Receptor Gene Rearrangements		
	IGH	*IGK*	*IGL*	*IGHV* SHM	*IGH-CCND1***	*IGH-BCL2*	*TRB*	*TRG*	*TRD*
IVS-0001						◆			
IVS-0004							◆	◆	
IVS-0007		◆	◆			◆			
IVS-0008*	❍						◆	◆	
IVS-0009							◆	◆	
IVS-0010	◆	◆	◆		◆				
IVS-0013	◆	◆	◆						
IVS-0019	◆	◆							
IVS-0021		◆					◆	◆	◆
IVS-0024	◆	◆							
IVS-0029	◆	◆	◆						
IVS-0030 ‡	◆	◆		◆		◆			
IVS-0031	◆	◆				◆			
IVS-P002						◆			

◆ Gene rearrangement ❍ Partial *IGH* DH-JH rearrangement

*This control does not contain a complete *IGH* VH-JH rearrangement and may only be suitable for *IGH* DH-JH rearrangements.
**_IGH-CCND1_ was previously referred to as *BCL1/JH*

‡These controls can be used as SHM positive controls with ≥2% mutational rates compared to the germline sequence.

Tissue DNA

Standard Concentrations

The vast majority of our high-quality DNA controls, including sensitivity controls and panels, are supplied in aliquots of 100 µL and are adjusted to a final concentration of 200 µg/mL in 1/10 TE (1 mM Tris- HCl (pH 8.0), 0.1 mM EDTA). This diluent provides sufficient buffering capacity and EDTA to protect the DNA without interfering with the Mg^{2+} concentrations required for robust amplification reactions.

IVS-0000 Polyclonal Control DNA

Tissue DNA controls are extracted from normal, disease-free tissue and are tested extensively to ensure quality and reproducibility of your test results. IVS-0000 Polyclonal Control DNA consists of genomic DNA isolated from the tissue of normal human tonsils. This control represents an excellent negative control for gene rearrangements, chromosome translocations, and mutation tests and is included in all of our PCR DNA-based assay kits. This DNA is supplied at a volume of 100 µL and at a concentration of 200 µg/mL.

Catalog #	Description
4-092-0010	IVS-0000 Polyclonal Control DNA*

Cell Line DNA

Reliable Positive Controls

Cell Line DNA controls are extracted from established cell lines grown under cell culture conditions recommended by the supplier. Our controls are tested extensively to ensure quality and reproducibility of your test results. Please note, these controls are for qualitative use only.

Note: n/c is used to indicate that the control has not been fully characterized; there may be additional rearrangements, translocations or mutations associated with the control.

Standard Concentrations

Our high-quality DNA controls, including our sensitivity panels, are supplied in aliquots of 100 µL and are adjusted to a final concentration of 200 µg/mL in 1/10 TE (1 mM Tris-HCl (pH 8.0), 0.1 mM EDTA). This diluent provides sufficient buffering capacity and EDTA to protect the DNA controls without interfering with the Mg^{2+} concentrations required for robust amplification reactions. DNA dilutions are diluted volume to volume (v/v) in our negative control DNA, IVS-0000 Polyclonal Control DNA.

IVS-0001 Clonal Control DNA

IVS-0001 Clonal Control DNA can be used as a positive control for:

Gene Rearrangements:	n/c
Chromosome Translocations:	*IGH-BCL2* t(14;18) mcr
Mutations:	n/c

Catalog #	Description
4-088-0010	100% IVS-0001 Clonal Control DNA*

IVS-0004 Clonal Control DNA

IVS-0004 Clonal Control DNA can be used as a positive control for:

Gene Rearrangements:	*TRB, TRG*
Chromosome Translocations:	n/c
Mutations:	n/c

This control is available as several ready-to-use dilutions generated by diluting the positive control volume to volume (v/v) into DNA characterized as negative.

Catalog #	Description
4-088-0190	100% IVS-0004 Clonal Control DNA*
4-088-0210	20% IVS-0004 Clonal Control DNA
4-088-0220	10% IVS-0004 Clonal Control DNA
4-088-0230	5% IVS-0004 Clonal Control DNA

IVS-0007 Clonal Control DNA

IVS-0007 Clonal Control DNA can be used as a positive control for:

Gene Rearrangements:	*IGH, IGK, IGL*
Chromosome Translocations:	*IGH-BCL2* t(14;18) Mbr
Mutations:	n/c

This control is also available as several ready-to-use dilutions into a standard negative control as listed in the table below.

Catalog #	Description
4-088-0370	100% IVS-0007 Clonal Control DNA*
4-088-0390	20% IVS-0007 Clonal Control DNA
4-088-0400	10% IVS-0007 Clonal Control DNA
4-088-0410	5% IVS-0007 Clonal Control DNA
4-088-0420	1% IVS-0007 Clonal Control DNA

* These controls are general purpose reagents (GPRs). All others are research use only (RUO).

Cell Line DNA

IVS-0008 Clonal Control DNA

IVS-0008 Clonal Control DNA can be used as a positive control for:

Gene Rearrangements: *IGH* DH-JH‡, *TRB, TRG*
Chromosome Translocations: n/c
Mutations: n/c

This control is also available as several ready-to-use dilutions into a standard negative control as listed in the table below.

Catalog #	Description
4-088-0430	100% IVS-0008 Clonal Control DNA*
4-088-0470	5% IVS-0008 Clonal Control DNA
4-088-0480	1% IVS-0008 Clonal Control DNA

IVS-0009 Clonal Control DNA

IVS-0009 Clonal Control DNA can be used as a positive control for:

Gene Rearrangements: *TRB, TRG*
Chromosome Translocations: n/c
Mutations: n/c

This control is also available as several ready-to-use dilutions into a standard negative control as listed in the table below.

Catalog #	Description
4-088-0490	100% IVS-0009 Clonal Control DNA*
4-088-0500	30% IVS-0009 Clonal Control DNA
4-088-0520	10% IVS-0009 Clonal Control DNA
4-088-0530	5% IVS-0009 Clonal Control DNA

IVS-0010 Clonal Control DNA

IVS-0010 Clonal Control DNA can be used as a positive control for:

Gene Rearrangements: *IGH, IGK, IGL*
Chromosome Translocations: *IGH-BCL1* t(11;14)
Mutations: n/c

This control is also available as several ready-to-use dilutions into a standard negative control as listed in the table below.

Catalog #	Description
4-088-0550	100% IVS-0010 Clonal Control DNA*
4-088-0560	30% IVS-0010 Clonal Control DNA
4-088-0580	10% IVS-0010 Clonal Control DNA
4-088-0590	5% IVS-0010 Clonal Control DNA

IVS-0013 Clonal Control DNA

IVS-0013 Clonal Control DNA can be used as a positive control for:

Gene Rearrangements: *IGH, IGK, IGL*
Chromosome Translocations: n/c
Mutations: n/c

Catalog #	Description
4-088-0730	100% IVS-0013 Clonal Control DNA*

IVS-0019 Clonal Control DNA

IVS-0019 Clonal Control DNA can be used as a positive control for:

Gene Rearrangements: *IGH, IGK*
Chromosome Translocations: n/c
Mutations: n/c

This control is also available as several ready-to-use dilutions into a standard negative control as listed in the table below.

Catalog #	Description
4-088-1090	100% IVS-0019 Clonal Control DNA*
4-088-1100	30% IVS-0019 Clonal Control DNA
4-088-1110	20% IVS-0019 Clonal Control DNA
4-088-1120	10% IVS-0019 Clonal Control DNA
4-088-1130	5% IVS-0019 Clonal Control DNA
4-088-1140	1% IVS-0019 Clonal Control DNA

IVS-0021 Clonal Control DNA

IVS-0021 Clonal Control DNA can be used as a positive control for:

Gene Rearrangements: *TRB, TRD, TRG*
Chromosome Translocations: n/c
Mutations: n/c

Catalog #	Description
4-088-1210	100% IVS-0021 Clonal Control DNA*
4-088-1220	30% IVS-0021 Clonal Control DNA
4-088-1230	20% IVS-0021 Clonal Control DNA
4-088-1240	10% IVS-0021 Clonal Control DNA
4-088-1250	5% IVS-0021 Clonal Control DNA
4-088-1260	1% IVS-0021 Clonal Control DNA

IVS-0024 Clonal Control DNA

IVS-0024 Clonal Control DNA can be used as a positive control for:

Gene Rearrangements: *IGH, IGK*
Chromosome Translocations: n/c
Mutations: n/c

This control is also available as a 5% ready-to-use dilution into a standard negative control as listed in the table below.

Catalog #	Description
4-088-1390	100% IVS-0024 Clonal Control DNA*
4-088-1430	5% IVS-0024 Clonal Control DNA

* These controls are general purpose reagents (GPRs). All others are research use only (RUO).

‡ This control does not contain a complete *IGH* VH-JH rearrangement and may only be suitable for *IGH* DH-JH rearrangements.

Cell Line DNA

IVS-0029 Clonal Control DNA

IVS-0029 Clonal Control DNA can be used as a positive control for:

Gene Rearrangements: *IGH, IGK, IGL*
Chromosome Translocations: n/c
Mutations: n/c

This control is also available as several ready-to-use dilutions into a standard negative control as listed in the table below.

Catalog #	Description
4-088-1690	100% IVS-0029 Clonal Control DNA*
4-088-1700	30% IVS-0029 Clonal Control DNA
4-088-1730	5% IVS-0029 Clonal Control DNA

IGH SHM Positive Control DNA

IVS-0028 Clonal Control DNA can be used as a positive control for:

Gene Rearrangements: *IGH*
Chromosome Translocations: n/c
Mutations: *IGH* SHM

Catalog #	Description
4-088-0008	*IGH* SHM Positive Control DNA‡

IVS-0030 Clonal Control DNA

IVS-0030 Clonal Control DNA can be used as a positive control for:

Gene Rearrangements: *IGH, IGK*
Chromosome Translocations: *IGH-BCL2* t(14;18) Mbr
Mutations: n/c

This control is also available as several ready-to-use dilutions into a standard negative control as listed in the table below.

Catalog #	Description
4-088-1750	100% IVS-0030 Clonal Control DNA*
4-088-1760	30% IVS-0030 Clonal Control DNA
4-088-1770	20% IVS-0030 Clonal Control DNA
4-088-1780	10% IVS-0030 Clonal Control DNA
4-088-1790	5% IVS-0030 Clonal Control DNA
4-088-1800	1% IVS-0030 Clonal Control DNA

IVS-0031 Clonal Control DNA

IVS-0031 Clonal Control DNA can be used as a positive control for:

Gene Rearrangements: *IGH, IGK*
Chromosome Translocations: *IGH-BCL2* t(14;18) mcr
Mutations: n/c

This control is also available as several ready-to-use dilutions into a standard negative control as listed in the table below.

Catalog #	Description
4-088-1810	100% IVS-0031 Clonal Control DNA*
4-088-1840	10% IVS-0031 Clonal Control DNA
4-088-1860	1% IVS-0031 Clonal Control DNA

Plasmid DNA

Reliable Positive Controls

Plasmid DNA controls are engineered to test positive for a specific chromosome translocation or mutation using our assay master mixes. The plasmid is cloned in standard bacterial culture conditions recommended by the supplier. Our controls are tested extensively to ensure quality and reproducibility of your test results. Please note, these controls are for qualitative use only.

Note: These controls are assay-specific and may not generate products with other assays.

Standard Concentrations

Plasmid IVS-P002 is diluted in IVS-0000 Polyclonal Control DNA (at 200 µg/mL in 1/10 TE) to mimic a normal background population and is for Research Use Only.

IVS-P002 Clonal Control DNA

IVS-P002 Clonal Control DNA can be used as a *IGH-BCL2* t(14;18) (3′ Mbr-JH) positive control for the *BCL2/*JH Tube B master mix included in our *BCL2/*JH Translocation Assay.

Plasmid Concentration: 100 µL @ 1600 pg/mL
Diluent: IVS-0000 Polyclonal Control DNA
Plasmid Size: 4.06 kb

Catalog #	Description
4-090-0070	IVS-P002 Clonal Control DNA*

* These controls are general purpose reagents (GPRs). All others are research use only (RUO).

‡ This control DNA does not contain a complete *IGH* VH-JH rearrangement and may only be suitable for *IGH* DH-JH rearrangements.

RNA Controls

Quick Reference for RNA Controls

Reliable Assay Controls

Our RNA controls are extracted from well characterized cell lines grown under standard and carefully controlled culture conditions. The controls are tested to ensure linearity and reproducible results. Since this RNA is extracted from cell lines, these controls can be used with any of the standard housekeeping genes.

Standard Concentrations

Each RNA single control tube (as separate control tube, RNA sensitivity panel and proficiency panel) is supplied in aliquots of 100 µL at a final concentration of 400 µg/mL in water. Each *BCR/ABL* RNA dilution set member is supplied in aliquots of 50 µL at a final concentration of 400 µg/mL in water. To ensure maximum stability, the dilution set should be stored at -65 °C to -85 °C and the number of freeze-thaw cycles should be kept to a minimum.

RNAs positive for chromosome translocations

Chromosome Translocation	Clonal Control RNA	Chromosome Translocation	Clonal Control RNA
BCR-ABL1 t(9;22) p210 e13a2 (b2a2)	IVS-0003	*CBFB-MYH11* inv(16)	IVS-0015
BCR-ABL1 t(9;22) p210 e14a2 (b3a2)	IVS-0011	*E2A-PBX1* t(1;19)(q23;p13)	IVS-0002
BCR-ABL1 t(9;22) p190 e1a2	IVS-0032	*PML-RARA* t(15;17)(q22;q11)	IVS-0020

RNAs negative for chromosome translocations

IVS-0035 can be used as a negative control for our *BCR/ABL* t(9;22) and *PML/RARA* t(15;17) Translocation Assays.

IVS-0035 may be used as a negative control for other chromosome translocation assays, or diluents for other chromosome translocation positive controls. Please do not hesitate to contact us at sales@invivoscribe.com so we can evaluate whether this control may work for your testing needs.

Cell Line RNA

Reliable Positive and Negative Controls

Cell Line RNA controls are extracted from established cell lines grown under cell culture conditions recommended by the supplier. Our controls are tested extensively to ensure quality and reproducibility of your test results. Please note, these controls are for qualitative use only.

Standard Concentrations

Our GMP-manufactured high-quality RNA controls, including sensitivity controls and proficiency panel samples, are supplied in aliquots of 100 µL and are adjusted to a final concentration of 400 µg/mL in RNase-free glass-distilled water. The pH of distilled water is slightly acidic; this protects the RNA from hydrolysis. RNA dilutions are diluted volume to volume in our negative control RNA, IVS-0035 Clonal Control RNA.

IVS-0011 Clonal Control RNA

IVS-0011 Clonal Control RNA can be used as a positive control for the chromosome translocation: *BCR-ABL1* t(9;22) p210 e14a2 (b3a2).

Catalog #	Description
4-089-0910	IVS-0011 Clonal Control RNA*
4-089-0920	10^{-1} IVS-0011 Clonal Control RNA
4-089-0930	10^{-2} IVS-0011 Clonal Control RNA
4-089-0940	10^{-3} IVS-0011 Clonal Control RNA
4-089-0950	10^{-4} IVS-0011 Clonal Control RNA
4-089-0960	10^{-5} IVS-0011 Clonal Control RNA

IVS-0002 Clonal Control RNA

IVS-0002 Clonal Control RNA can be used as a positive control for the chromosome translocation: *E2A-PBX1* t(1;19) (q23;p13).

Catalog #	Description
4-089-0100	IVS-0002 Clonal Control RNA*

IVS-0003 Clonal Control RNA

IVS-0003 Clonal Control RNA can be used as a positive control for the chromosome translocation: *BCR-ABL1* t(9;22) p210 e13a2 (b2a2).

This control is also available as several ready-to-use dilutions into a standard negative control as listed in the table below.

Catalog #	Description
4-089-0190	IVS-0003 Clonal Control RNA*
4-089-0200	10^{-1} IVS-0003 Clonal Control RNA
4-089-0210	10^{-2} IVS-0003 Clonal Control RNA
4-089-0220	10^{-3} IVS-0003 Clonal Control RNA
4-089-0230	10^{-4} IVS-0003 Clonal Control RNA
4-089-0240	10^{-5} IVS-0003 Clonal Control RNA
4-089-0250	10^{-6} IVS-0003 Clonal Control RNA

IVS-0015 Clonal Control RNA

IVS-0015 Clonal Control RNA can be used as a positive control for the chromosome translocation: *CBFB-MYH11* inv(16).

This control is also available as several ready-to-use dilutions into a standard negative control as listed in the table below.

Catalog #	Description
4-089-1270	IVS-0015 Clonal Control RNA*

IVS-0020 Clonal Control RNA

IVS-0020 Clonal Control RNA can be used as a positive control for the chromosome translocation: *PML-RARA* t(15;17) L-Form.

This control is also available as several ready-to-use dilutions into a standard negative control as listed in the table below.

Catalog #	Description
4-089-1720	IVS-0020 Clonal Control RNA*
4-089-1730	10^{-1} IVS-0020 Clonal Control RNA
4-089-1740	10^{-2} IVS-0020 Clonal Control RNA
4-089-1750	10^{-3} IVS-0020 Clonal Control RNA
4-089-1760	10^{-4} IVS-0020 Clonal Control RNA

IVS-0032 Clonal Control RNA

IVS-0032 Clonal Control RNA can be used as a positive control for the chromosome translocation: *BCR-ABL1* t(9;22) p190 e1a2.

This control is also available as several ready-to-use dilutions into a standard negative control as listed in the table below.

Catalog #	Description
4-089-2800	IVS-0032 Clonal Control RNA*
4-089-2810	10^{-1} IVS-0032 Clonal Control RNA
4-089-2820	10^{-2} IVS-0032 Clonal Control RNA
4-089-2830	10^{-3} IVS-0032 Clonal Control RNA
4-089-2840	10^{-4} IVS-0032 Clonal Control RNA
4-089-2850	10^{-5} IVS-0032 Clonal Control RNA
4-089-2860	10^{-6} IVS-0032 Clonal Control RNA

IVS-0035 Clonal Control RNA

IVS-0035 Clonal Control RNA can be used as a negative control for our *BCR/ABL1* t(9;22) and *PML/RARa* t(15;17) Translocation Assays.

Catalog #	Description
4-089-3070	IVS-0035 Clonal Control RNA*

* These controls are general purpose reagents (GPRs). All others are research use only (RUO).

BCR1/ABL1 RNA Dilution Sets

Our *BCR/ABL* b2a2, b3a2, and e1a2 RNA Dilution Sets consist of RNA that has been extracted from *BCR-ABL1* expressing and *BCR-ABL1* negative cell lines. Each set is composed of several dilutions (10-1, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}) of the *BCR-ABL1* positive RNA diluted (v/v) into RNA purified from a cell line that does not contain a *BCR-ABL1* translocation. Also included in these sets is a 100% *BCR-ABL1* negative RNA.

The individual *BCR/ABL* b2a2, b3a2, and e1a2 RNA Dilution Sets can be used as reference and validation materials with assays that target the main transcripts of *BCR-ABL1* t(9;22) translocations: p210 (e13a2 (b2a2), e14a2 (b3a2), and p190 (e1a2). These products may be used as the following:

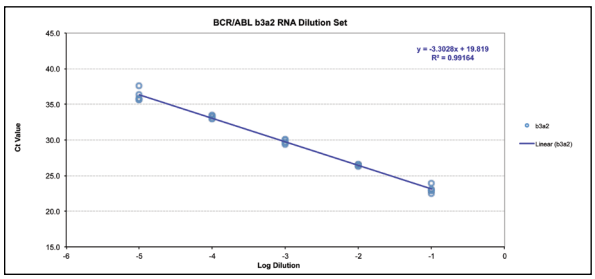
- Routine testing controls for cDNA synthesis, amplification and detection
- Controls to establish a standard reference curve
- Proficiency controls
- Sensitivity controls for specific target assays

Data

Plot of Ct values (5 replicates) for the 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} dilutions.

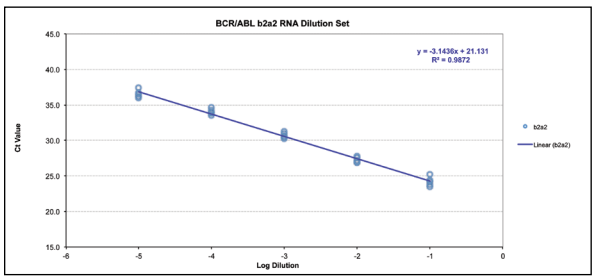
Ordering Information – e14a2 (b3a2)

Catalog #	Description
4-085-0210	*BCR/ABL* b3a2 RNA Dilution Set (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} dilutions and negative)



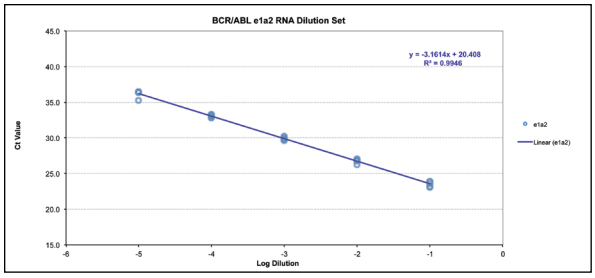
Ordering Information – e13a2 (b2a2)

Catalog #	Description
4-085-0310	*BCR/ABL* b2a2 RNA Dilution Set (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} dilutions and negative)



Ordering Information – e1a2

Catalog #	Description
4-085-0110	*BCR/ABL* e1a2 RNA Dilution Set (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} dilutions and negative)



Control Panels

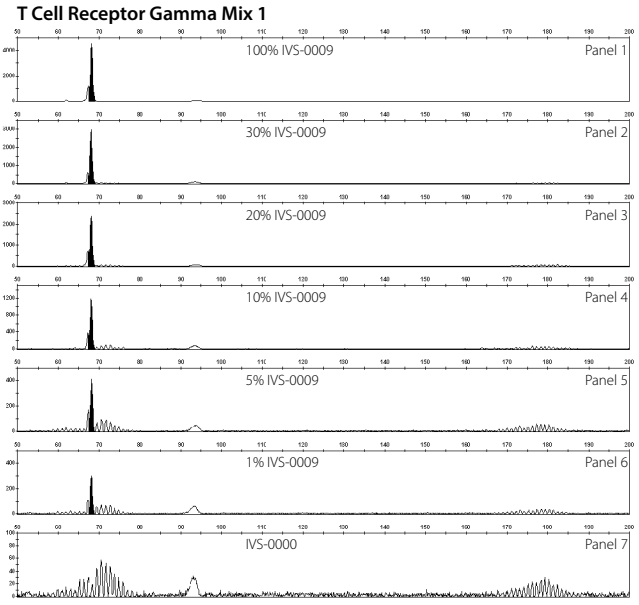
DNA and RNA Sensitivity Panels

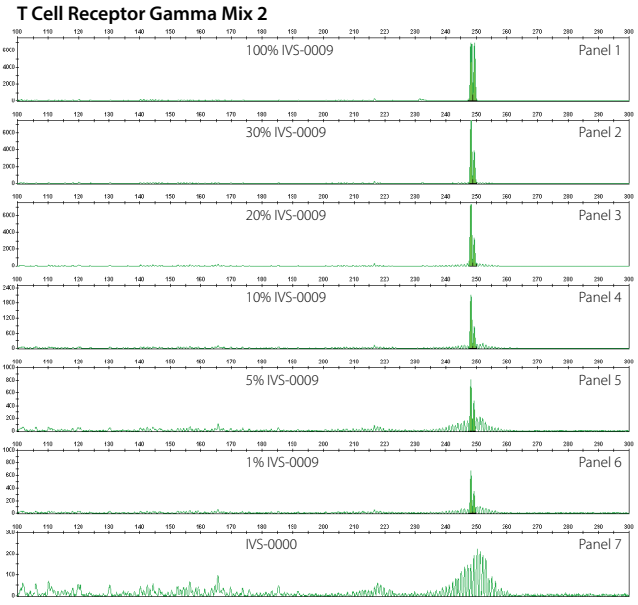
DNA Sensitivity Panels

DNA sensitivity panels are 6 member panels that consist of 100% clonal DNA extracted from a positive control cell line and 30%, 20%, 10%, 5%, and 1% dilutions of the positive clonal DNA diluted (v/v) into our standard negative control DNA, IVS-0000 Polyclonal Control DNA. Each tube contains 100 µL of DNA at a concentration of 200 µg/mL in 1/10 TE buffer (1 mM Tris-HCl pH 8.0, 0.1 mM EDTA). This diluent provides sufficient buffering capacity and EDTA to protect the DNA controls without interfering with the Mg^{2+} concentrations required for robust amplification reactions. Please note, these controls are for qualitative use only.

RNA Sensitivity Panels

RNA sensitivity panels are 7 member panels that consist of 100% clonal RNA extracted from a positive control cell line and 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} (1:10 – 1:1 000 000) dilutions of the positive clonal RNA diluted (v/v) into our standard negative control RNA, IVS-0035 Clonal Control RNA. Each tube contains 100 µL of RNA at 400 µg/mL in RNase-free glass-distilled water. The pH of distilled water is slightly acidic thereby protecting the RNA from hydrolysis. Please note, these controls are for qualitative use only.





This data was generated testing a Sensitivity Panel for IVS-0009 Clonal Control DNA using the master mixes listed. PCR products were run on an ABI 3130xL capillary electrophoresis instrument for differential fluorescence detection and data analyses. Panel 7 shows the polyclonal Gaussian distributions expected from our negative control IVS-0000 Polyclonal Control DNA. Data in the other panels are tests of positive control samples at the dilutions indicated. Clonal peaks (highlighted) are clearly evident in all of the positive sample panels.

DNA Sensitivity Panels

Catalog #	Description	Can be used as a positive control for:
4-086-0040	Sensitivity Panel for IVS-0004 Clonal Control DNA	*TRB, TRG*
4-086-0070	Sensitivity Panel for IVS-0007 Clonal Control DNA	*IGH, IGK, IGL, IGH-BCL2* t(14;18) Mbr
4-086-0090	Sensitivity Panel for IVS-0009 Clonal Control DNA	*TRB, TRG*
4-086-0100	Sensitivity Panel for IVS-0010 Clonal Control DNA	*IGH, IGK, IGL, IGH-CCND1* t(11;14)
4-086-0190	Sensitivity Panel for IVS-0019 Clonal Control DNA	*IGH, IGK*
4-086-0210	Sensitivity Panel for IVS-0021 Clonal Control DNA	*TRB, TRD, TRG*
4-086-0300	Sensitivity Panel for IVS-0030 Clonal Control DNA	*IGH, IGK, IGH-BCL2* t(14;18) Mbr

RNA Sensitivity Panels

Catalog #	Description	Can be used as a positive control for:
4-087-0030	Sensitivity Panel for IVS-0003 Clonal Control RNA	*BCR-ABL1* t(9;22) p210 e13a2 (b2a2)
4-087-0110	Sensitivity Panel for IVS-0011 Clonal Control RNA	*BCR-ABL1* t(9;22) p210 e14a2 (b3a2)
4-087-0150	Sensitivity Panel for IVS-0015 Clonal Control RNA	*CBFB/MYH11* inv16
4-087-0200	Sensitivity Panel for IVS-0020 Clonal Control RNA	*PML-RARA* t(15;17) L-form
4-087-0320	Sensitivity Panel for IVS-0032 Clonal Control RNA	*BCR-ABL1* t(9;22) p190 e1a2

Proficiency Panel for *BCR-ABL1* t(9;22)

This 10 member panel consists of 100% clonal control RNA extracted from three *BCR-ABL1* positive cell lines as well as 10^{-2} (1:100) and 10^{-4} (1:10,000) dilutions (v/v) of these positive RNAs diluted into a normal (*BCR-ABL1* negative) control RNA, IVS-0035 Clonal Control RNA. A sample of 100% IVS-0035 Clonal Control RNA is also included. All three cell lines (IVS-0003, IVS-0011, and IVS-0032) carry a t(9;22) translocation. One of the cell lines, IVS-0032, encodes for p190-type (ALL-associated) transcript with e1a2 junctions. The other two cell lines both encode for p210-type (CML-associated) transcripts. One of the p210-type translocations, IVS-0003, harbors a e13a2 (b2a2) junction and the other, IVS-0011, harbors a e14a2 (b3a2) junction. This proficiency panel is used to validate tests that identify *BCR-ABL1* t(9;22) translocations and is designed to be used with the *BCR/ABL* t(9;22) Translocation Assay Kits.

Ordering Information

Catalog #	Description
4-310-0100	Proficiency Panel for the *BCR/ABL* t(9;22) Translocation Assay

Master Mix Controls

These master mixes serve as control for many of our DNA assays to ensure that sample DNA is of sufficient quality and integrity to generate a valid result.

Amplification Control Master Mix

Our Amplification Control master mix targets the *HLA*-DQa locus and generates a product of 235 basepairs in size from human genomic DNA. This control is available in unlabeled (for Gel Detection) and fluorescence labeled format (for ABI Fluorescence Detection, 6FAM).

Catalog #	Description
2-096-0010	Amplification Control Master Mix - Unlabeled*
2-096-0011	Amplification Control Master Mix - 6FAM*

Specimen Control Size Ladder

Our Specimen Control Size Ladder master mix targets four different housekeeping genes producing products of approximately 100, 200, 300, 400, and 600 basepairs in size to ensure that the quality and quantity of the sample DNA is adequate to yield a valid result with the specific assay(s).

This master mix is based on the BIOMED-2 Concerted Action BMH4-CT98-3936 from the EuroClonality Group and is available for Gel Detection (unlabeled) or ABI detection (labeled with 6FAM).

Catalog #	Description
2-096-0020	Specimen Control Size Ladder - Unlabeled*
2-096-0021	Specimen Control Size Ladder - 6FAM*

* These master mixes are general purpose reagents (GPRs).

Reagents

ABI Detection Reagents

Reagents for ABI Fluorescence Detection

Invivoscribe also offers highly deionized (Hi-Di) Formamide with and without ROX size standards for ABI fluorescence detection with the ABI 310 or 3100 series. Hi-Di Formamide is used to stabilize single strands of denatured PCR amplicons. The ROX size standards are fluorescent labeled DNA standards which cover the 50 to 400 basepair size range. Sizes of the individual standards are: 50, 60, 90, 100, 120, 150, 160, 180, 190, 200, 220, 240, 260, 280, 290, 300, 320, 340, 360, 380, and 400 basepairs.

For samples tested on an ABI 310 or 3100 series, we recommend using 10 µL of the Hi-Deionized Formamide with ROX Size Standards mixture for each microliter of PCR product. Please note that the ABI 310 and 3100 series require different concentrations of ROX size standards and the different Hi-Deionized Formamide with ROX Size Standards cannot be used interchangeably.

For samples tested on an ABI 3500 series, GeneScan™ 600® LIZ dye Size Standard v2.0 can be purchased from Thermo Fisher Scientific.

Ordering Information

Catalog #	Description
6-098-0051	Hi-Deionized Formamide with ROX Size Standard (ABI 310), 1 mL
6-098-0061	Hi-Deionized Formamide with ROX Size Standard (ABI 3100), 1 mL
Available through Thermo Fisher Scientific®: 4408399	GeneScan™ 600 LIZ® dye v2.0 Standard (ABI 3500), 800 reactions

Enzymes

EagleTaq DNA Polymerase

Note: This product is for sale and use in the European Economic Area only. It is not to be resold or transferred to another party. See also Notice on page 112.

Hot Start Taq DNA Polymerase

EagleTaq DNA Polymerase can be used to obtain highly specific and sensitive PCR amplification products. This enzyme has been proven to minimize extension of non-specifically bound primers. Obtain reliable results by using the gold standard of hot start polymerases for robust performance.

Ordering Information

Catalog #	Description
6-097-0100	EagleTaq DNA Polymerase 1000 U, 5 U/µL

Warranty and Liability

Invivoscribe Technologies, Inc. (Invivoscribe®) is committed to providing the highest quality products. Invivoscribe® warrants that the products meet or exceed the performance standards described in the Instructions For Use, as to products with such an insert. If a product is covered by product specifications and does not perform as specified, our policy is to replace the product or credit the full purchase price. No other warranties of any kind, expressed or implied, are provided by Invivoscribe®. Invivoscribe® liability shall not exceed the purchase price of the product. Invivoscribe shall have no liability for direct, indirect, consequential, or incidental damages arising from the use, results of use, or inability to use its products; product efficacy under purchaser controlled conditions in purchaser's laboratory must be established and continually monitored through purchaser defined and controlled processes including but not limited to testing of positive, negative, and blank controls every time a sample is tested. Ordering, acceptance, and use of product constitutes purchaser acceptance of sole responsibility for assuring product efficacy and purchaser agreement to the limitation of liability set forth in this paragraph.

Custom Products

Customized Products to Meet Your Needs

The Invivoscribe team of experts can help develop your ideas into customized products. Allow us to partner with you to take a basic concept through design, development, validation, regulatory approval (if applicable), and release. For more information, please call our San Diego office at +1 858.224.6600 or send an e-mail to marketing@invivoscribe.com.

Custom Designed Assays

In response to the FDA announcing its intention to dramatically expand its regulatory oversight of laboratory developed tests (LDTs), Invivoscribe is partnering with laboratories worldwide to help facilitate the conversion of LDTs into FDA-cleared assays, as we know the barriers to bringing new assays online are often the availability of resources and the cost of validation.

By leveraging the power of our regulatory expertise, provided through each milestone, we can help ensure safety, efficacy and quality. Our customizable reagent manufacturing capabilities can reduce your LDT costs and lead to higher-quality testing.

To date, we have partnered with more than 40 laboratories around the world to develop, validate, and launch a variety of molecular products. A number of these partnerships have also led to the release of US and CE-marked *in vitro* diagnostic products and services. Learn how Invivoscribe can help you develop assays for new products, services, and novel applications.

Custom Controls and Validation Panels

We offer a large selection of well-characterized DNA and RNA controls that are used to define the performance characteristics of a wide variety of molecular reagents. To address your specific requirements, we can partner with you to design, validate, and provide custom controls and validation panels. If necessary, we are willing to acquire, characterize, and engineer custom controls for your specific application. We can produce DNA, RNA, or cDNA at any specified concentration, dilution, or volume. Please contact us with your requirements and we will be happy to provide controls to suit your needs.

Invivoscribe is a Comprehensive Partner for Companion Diagnostic Development

From biomarker identification through commercialization, Invivoscribe has expertise at every stage of companion diagnostics development.

- **Discovery & Patient Stratification:** We offer comprehensive gene panels to identify biomarkers and define patient populations, thus reducing development costs and improving the success of clinical trials.
- **Clinical Trials:** Our network of global laboratories accelerates sample acquisition and harmonizes testing to ensure accurate results.
- **Regulatory Approval:** Our in-house experts have experience seeking approval with global agencies.
- **Commercialization:** Our cGMP manufacturing expertise and distribution channels allow approved CDx to reach all global markets.

Invivoscribe is an ISO 13485-accredited and FDA/CDRH registered medical device manufacturer with a long record of successful partnerships. We are the industry-leading assay and software development company, providing full QSR design control and a complete range of cGMP manufactured assays, controls, reagents, and services to CLIA-accredited clinical laboratory and pharmaceutical communities.

Please contact us at +1 858.224.6600 or marketing@invivoscribe.com for more details about partnering with Invivoscribe for the development and manufacturing of companion diagnostics, *in vitro* diagnostics, molecular reagents, and/or nucleic acid controls.

Warranty and Liability

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NGS Cancer Panels
Laboratory Services

Comprehensive panels aimed at promoting a further understanding of patients' clinical responses and outcomes

Next-Generation Sequencing (NGS) is rapidly replacing more traditional methods of cancer genotyping with numerous new mutations and gene expression signatures having been identified, allowing us to better understand the molecular heterogeneity of hematologic diseases and to better stratify and assess risk for cancer patients.

Using these molecular tools, it has become evident that leukemias, lymphomas and oncologic diseases are characterized by a remarkable genetic heterogeneity, with individual patients presenting with a distinct and almost unique combination of chromosome changes and somatically acquired gene mutations.

Leading the way on molecular testing of hematologic malignancies for more than two decades, Invivoscribe has developed three broad gene panels that aim to identify clinically actionable, pathogenic, and potentially pathogenic mutations in relevant sets of genes. Namely, our MyAML®, MyHEME® and MyMRD™ panels, combined with our custom bioinformatics pipeline, MyInformatics™, interrogate clinical samples up to a depth of 10^{-3} variant frequency, which provides information on prominent somatic driver mutations, subclonal architecture, mutation tracking and MRD status.

These assays are currently being provided as services through Invivoscribe fully-owned subsidiary, Laboratories for Personalized Molecular Medicine (LabPMM).

San Diego, CA, USA

CLIA, CAP, ISO 15189-accredited & New York State licensed

Martinsried, Germany

Accredited by DAkkS to ISO 15189
CLIA/CAP accreditation (in progress)

Tokyo, Japan

ISO 15189, CLIA/CAP accreditations (in progress)

Shanghai, China

ISO 15189, CLIA/CAP accreditations planned

MyAML®

MyAML® - NGS Panel

Intended Use

The MyAML is a CLIA validated assay which identifies clinically actionable, pathogenic, and potentially pathogenic mutations in 194 genes associated with AML. Using the latest version in Next-Generation Sequencing (NGS) chemistry, MyAML identifies all somatic mutations, large and small insertions/deletions and translocations under NCCN/ELN guidelines, as well as novel somatic variants that may have prognostic significance for AML.

The MyAML gene panel is aimed at promoting a further understanding of AML patients' clinical responses and outcomes.

Summary and Explanation of the Test

Using customized design, the coding regions and potential genomic breakpoints within known somatic gene fusions are sequenced to an average depth of coverage of 1000x. By utilizing long read lengths, the assay accurately detects and characterizes the breakpoints of structural variants and gene fusions, often with single base pair precision. In addition, these long reads enhance the ability to identify both the insertion site and DNA content of large internal tandem duplications. Coupling comprehensive gene coverage with enhanced depth of coverage, long read lengths, and the power of our robust MyInformatics™ annotation software and bioinformatics database, MyAML identifies the underlying somatic mutations that are present in as low as 5% allelic frequency. The data and report include single base resolution of the genomic breakpoint and sequences of mutations, which facilitates both minimal residual disease testing and temporal and longitudinal studies.

Background

It is becoming increasingly evident that understanding the clonal architecture of AML patients is vital for successful treatments[1]. It is likely that many different mutations, epigenetic aberrations or downstream abnormalities can generate the same clinical picture. However these differences are responsible for the variable responses observed with therapy, which is a major feature in patients with AML[2]. Therefore, since varied somatic mutations affect patient outcomes, conventional genotyping is no longer the most suitable method for screening patients.

Screening with MyAML allows generating treatment decisions based on knowing all relevant mutations in both the prevalent clone and those in 'secondary' or 'tertiary' clones that may become the new prominent clone leading to remission.

Reference

1. Döhner K et al. Intermediate-risk acute myeloid leukemia therapy: current and future. *Hematology Am Soc Hematol Educ Program* 1:34-43 (2014).
2. Estey EH, Acute myeloid leukemia: 2014 update on risk-stratification and management. *Am J Hematol* 89:1063-81 (2014).

Specimen Requirements

1. 5 mL of peripheral blood in Heparin, EDTA or ACD
2. 3 mL of bone marrow in Heparin, EDTA or ACD
3. Cell Pellets in cell culture media or buffered solutions without fixatives
4. 1 µg of purified, high quality genomic DNA

Turnaround Time

7-10 business days

Shipping Conditions

Ambient or Cool; Do not freeze

Storage Conditions

Room Temp up to 72 hours / 4 °C up to 7 days

MyAML Gene List

Structural Rearrangements Under NCCN/ELN Guidelines

Inv(16) t(16;16) t(8;21) t(15;17) +8 t(9;11) -5 5q- -7 7q- 11q23 inv(3) t(3;3) t(6;9) t(9;22)

These regions also include genes from the "fusions and gene rearrangements" below.

Fusions and Gene Rearrangements (36 genes)

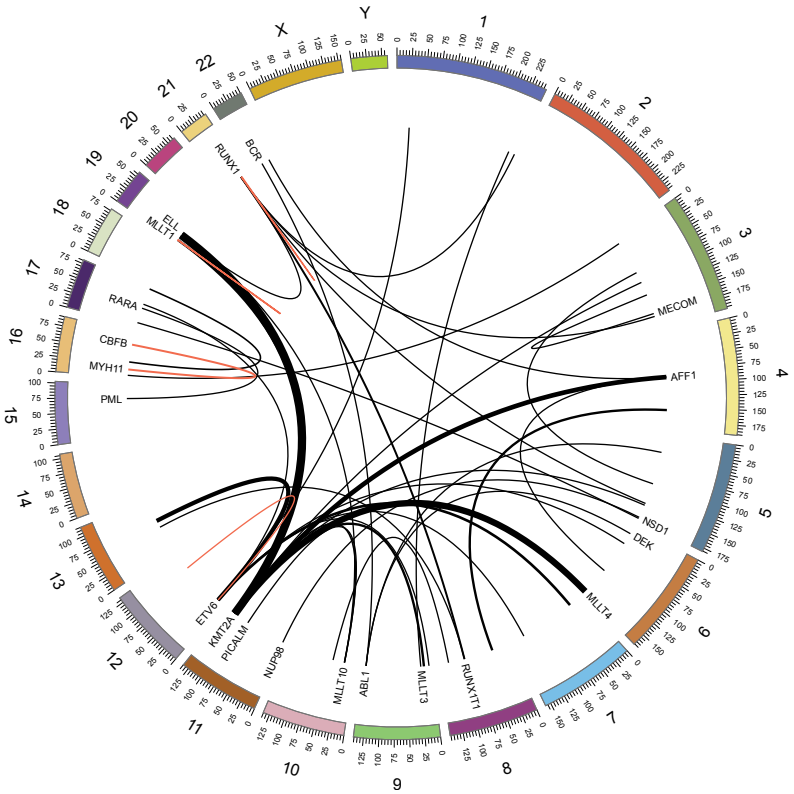
Including 5'UTRs, exons, recombination intron breakpoint hotspots, non-coding exons, and 3'UTRs.

ABL1 ADGRG7 AFF1 BCR CBFB CREBBP DEK EIF4E2 ELL ETV6 GAS6 GAS7 KAT6A KAT6B KMT2A MECOM MKL1 MLLT10 MLLT1 MLLT3 MLLT4 MYH11 NSD1 NUP214 NUP98 PICALM PML RARA RBM15 RPN1 RUNX1 RUNX1T1 SEPT5 SET TFG TMEM255B

Genes (158 genes)

Including 5'UTRs, exons, non-coding exons, and 3'UTRs.

ABCC1 ACVR2B ADRBK1 AKAP13 ANKRD24 ARID2 ARID4B ASXL1 ASXL2 ASXL3 BCOR BCORL1 BRINP3 BRPF1 BUB1 CACNA1E CBL CBX5 CBX7 CDC73 CEBPA CEP164 CPNE3 CSF1R CSTF2T CTCF CYLD DCLK1 DDX1 DDX23 DHX32 DIS3 DNAH9 DNMT1 DNMT3A DNMT3B DYRK4 EED EGFR EP300 EPHA2 EPHA3 ETV3 EZH2 FANCC FLT3 GATA1 GATA2 GFI1 GLI1 HDAC2 HDAC3 HNRNPK HRAS IDH1 IDH2 IKZF1 JAK1 JAK2 JAK3 JMJD1C KDM2B KDM3B KDM6A KDM6B KIT KMT2B KMT2C KRAS MAPK1 METTL3 MST1R MTA2 MTOR MXRA5 MYB MYC MYLK2 MYO3A NF1 NOTCH1 NOTCH2 NPM1 NRAS NRK OBSCN PAPD5 PAX5 PDGFRA PDGFRB PDS5B PDSS2 PHF6 PKD1L2 PLRG1 POLR2A PRDM16 PRDM9 PRKCG PRPF3 PRPF40B PRPF8 PTEN PTPN11 PTPN14 PTPRT RAD21 RBBP4 RBMX RPS6KA6 SAP130 SCML2 SETBP1 SETD2 SF1 SF3A1 SF3B1 SMC1A SMC3 SMC5 SMG1 SNRNP200 SOS1 SPEN SRRM2 SRSF2 SRSF6 STAG2 STK32A STK33 STK36 SUDS3 SUMO2 SUPT5H SUZ12 TCF4 TET1 TET2 THRB TP53 TRA2B TRIO TTBK1 TYK2 TYW1 U2AF1 U2AF1L4 U2AF2 UBA3 WAC WAPAL WEE1 WNK3 WNK4 WT1 ZBTB33 ZBTB7B ZRSR2



Above: Circos plot depicting a subset of translocations and inversions identified using MyAML translocations (black) and inversions (red) are depicted by curved lines (with width proportionate to the number of times the variant was observed) connecting two chromosomal regions together.

MyHEME®

MyHEME® - NGS Panel

Intended Use

The MyHEME assay is a RUO panel that identifies clinically actionable, pathogenic, and potentially pathogenic mutations in 932 targets (704 unique genes) known to, or predicted to contribute to hematological malignancies. Using the latest version in Next-Generation Sequencing (NGS) chemistry, MyHEME identifies all somatic mutations, large and small insertions/deletions and translocations under NCCN/ELN guidelines, as well as novel somatic variants that may have prognostic significance for hematological diseases.

The MyHEME gene panel is aimed at promoting a further understanding of hematologic cancer patients' clinical responses and outcomes.

Summary and Explanation of the Test

Using customized design, the coding and non-coding exons of 571 genes are sequenced to an average depth of coverage of 1000x. Long reads enhance the ability to identify indels, including large internal tandem duplications (ITDs). RNA sequencing of 371 genes identifies gene fusions and rearrangements, as well as provides gene expression information. Coupling comprehensive gene coverage with enhanced depth of coverage, long read lengths, and the power of our robust MyInformatics™ annotation software and bioinformatics database, MyHEME identifies the underlying somatic mutations that are present in as low as 5% allelic frequency. The data and report include sequences of mutations, which facilitates both minimal residual disease testing and temporal and longitudinal studies.

Background

Hematologic diseases are characterized by aberrant gene expression often resulting from specific genetic translocations or mutations that lead to unregulated signal transduction. These types of alterations allow classification of leukemias and lymphomas into specific subgroups and frequently suggest treatment strategies[1].

Screening with MyHEME allows generating treatment decisions based on knowing all relevant mutations in both the prevalent clone and those in 'secondary' or 'tertiary' clones that may become the new prominent clone leading to remission.

Reference

1. Gabrilove JL et al. (2001) Hematologic Malignancies: An Opportunity for Targeted Drug Therapy. *Oncologist 6 Suppl* 5:1-3

Specimen Requirements

DNA

- 5 mL of peripheral blood in Heparin, EDTA or ACD
- 3 mL of bone marrow in Heparin, EDTA or ACD
- Cell Pellets in cell culture media or buffered solutions without fixatives
- 1 µg of purified, high quality genomic DNA

RNA

- PAXgene tube for BMA or PBMC with 5 mL minimum blood volume

Turnaround Time

10-14 business days

Shipping Conditions

Ambient or Cool; Do not freeze

Storage Conditions

Room Temp up to 72 hours / 4 °C up to 7 days

MyHEME Genes Covered

Our MyHEME NGS panel includes analysis of genomic alterations in each of the genes listed below by characterizing the entire coding sequence of 571 genes, and utilizing RNA sequencing to analyze 371 genes known to be altered in various human hematologic malignancies.

DNA Targets (571 Genes)

ABI1 ABL1 ABL2 ACSL6 ACVR1C ACVR2B ADGRG7 ADNP ADRBK1 AFF1 AFF3 AFF4 AKAP13 AKT1 ALK ANKHD1 ANKRD26 ARAF ARHGAP26 ARHGEF12 ARID1A ARID1B ARID2 ARID4B ARID5B ARNT ASXL1 ASXL2 ASXL3 ATG2B ATIC ATM ATP2B3 ATR ATRX AXL B2M BAP1 BAX BAZ2A BCL10 BCL11A BCL11B BCL2 BCL3 BCL6 BCL7A BCL9 BCOR BCORL1 BCR BIRC3 BLM BMI1 BRAF BRCA1 BRCA2 BRD4 BRIP1 BRPF1 BTG1 BTK BUB1 C15orf65 CACNA1E CALR CAMK1G CAMKK1 CAMTA1 CARD11 CARS CASC5 CBFA2T3 CBFB CBL CBLB CBLC CBX5 CBX7 CCDC6 CCND1 CCND2 CCND3 CD274 CD70 CD79A CD79B CDC42EP1 CDH23 CDK11B CDK16 CDK6 CDKN1B CDKN2A CDKN2B CDKN2C CDX2 CEBPA CEBPE CHD2 CHIC2 CIITA CLP1 CLTC CLTCL1 CNOT3 CPNE3 CREBBP CRLF2 CSF1R CSF3R CSMD1 CSTF2T CTNNA1 CTNNB1 CUL1 CUX1 CXCR4 CYP1A1 DCLK1 DDR2 DDX1 DDX10 DDX11 DDX23 DDX3X DDX41 DDX6 DEK DHX15 DHX29 DHX32 DICER1 DIS3 DNAJC11 DNM2 DNMT3A DNMT3B DOT1L DTX1 DUSP2 DYRK4 ECT2L EED EGFR EGR1 EGR2 EIF4A2 EIF4E2 ELF4 ELL ELN EP300 EPHA10 EPHA2 EPHA3 EPOR EPS15 ERBB2 ERBB3 ERG ESCO2 ETNK1 ETV3 ETV6 EWSR1 EZH2 EZR FAM175A FAM46C FANCA FANCC FANCD2 FANCE FANCF FANCG FAS FAT3 FBXO11 FBXW7 FCGR2B FCRL4 FEV FGFR1 FGFR1OP FGFR2 FGFR3 FGFR4 FH FHIT FIP1L1 FLI1 FLT1 FLT3 FNBP1 FOXO1 FOXO3 FOXO4 FOXP1 FSIP2 FSTL3 FUBP1 FUS GAS6 GAS7 GATA1 GATA2 GATA3 GLI1 GMPS GNA13 GNAS GNB1 GPHN GSKIP HDAC1 HDAC2 HDAC3 HFE HIP1 HIST1H1B HIST1H1E HIST1H3B HIST1H4I HLF HLX HNRNPK HOXA11 HOXA13 HOXA9 HOXC11 HOXC13 HOXD11 HOXD13 HRAS HSP90AA1 HSP90AB1 ICE1 IDH1 IDH2 IKBKB IKZF1 IKZF2 IKZF3 IKZF4 IL15 IL2 IL21R IL7R INPP5D INTS12 IRAK1 IRF1 IRF4 IRF8 ITK ITPKB JAK1 JAK2 JAK3 JMJD1C KAT6A KAT6B KDM2B KDM3B KDM5A KDM6A KDM8 KDR KDSR KIT KLHDC8B KLHL6 KMT2A KMT2B KMT2C KMT2D KRAS LASP1 LCK LCP1 LMO1 LMO2 LPP LRIG3 LRP1B LTB LUC7L2 LYL1 MAF MAFB MALT1 MAP2K1 MAP3K1 MAP3K13 MAPK1 MAU2 MDM2 MDM4 MDS2 MECOM MED1 MED12 MEF2B MET METTL3 MIR142 MIR155 MKL1 MLF1 MLLT1 MLLT10 MLLT11 MLLT3 MLLT4 MLLT6 MN1 MNX1 MPL MSI2 MSN MST1R MTA2 MTCP1 MTOR MUC1 MUTYH MYB MYC MYD88 MYH11 MYH9 MYLK2 MYO3A NACA NBN NCKIPSD NCOA2 NF1 NF2 NFKB2 NFKBIE NIN NIPBL NKAP NLRP2 NOTCH1 NOTCH2 NPM1 NRAS NRG1 NRK NSD1 NT5C2 NUMA1 NUP214 NUP98 NXF1 OLIG2 P2RY8 PAFAH1B2 PAK1 PALB2 PAX5 PBRM1 PBX1 PCLO PCM1 PCSK7 PDCD1LG2 PDE4B PDE4DIP PDGFB PDGFRA PDGFRB PDS5A PDS5B PEG3 PER1 PHF6 PICALM PIGA PIK3CA PIK3CD PIM1 PIP4K2A PLCG1 PLRG1 PML POLR2A POLR3B POT1 POU2AF1 POU2F2 PRDM1 PRDM16 PRDM9 PRF1 PRKCG PRKD3 PRPF3 PRPF8 PRRX1 PSIP1 PTCH1 PTEN PTPN11 PTPN14 PTPN2 PTPN5 PTPN6 PTPRC PTPRF PTPRT PYGL RABEP1 RAD21 RALGDS RANBP17 RAP1GDS1 RARA RASA2 RBBP4 RBM15 RBMX REC8 REL RELN RET RHOA RHOH RMI2 RNF213 RNF217-AS1 ROBO1 RPL10 RPL22 RPL5 RPN1 RPS14 RPS15 RPS2 RPS6KA6 RPS6KB2 RUNX1 RUNX1T1 S1PR2 SAMHD1 SAP130 SBDS SCML2 SEPT5 SEPT6 SEPT9 SET SETBP1 SETD1A SETD2 SETDB1 SF3B1 SH2B3 SH2D1A SH3GL1 SKIV2L2 SMARCA2 SMARCA4 SMARCB1 SMC1A SMC3 SMC5 SMG1 SMO SNRNP200 SNX29 SNX7 SOCS1 SOS1 SP140 SPECC1 SPEN SPI1 SPOP SRP72 SRRM2 SRSF2 SRSF3 SRSF6 STAG1 STAG2 STAT3 STAT5B STAT6 STIL STK11 STK32A STK33 STK36 SUDS3 SUPT5H SUZ12 SYK SYNE1 TAF15 TAL1 TAL2 TBL1XR1 TCF3 TCL1A TCL6 TERC TERT TET1 TET2 TFG TFPT TFRC TGDS TLR2 TLX1 TLX3 TMEM255B TNF TNFAIP3 TNFRSF14 TNFRSF17 TOP1 TP53 TP63 TPM3 TPM4 TPMT TRA2B TRAF3 TRIM24 TRIO TRIP11 TSC1 TSC2 TTBK1 TTL TYK2 TYRO3 U2AF1 U2AF1L4 U2AF2 UBR5 VHL WAC WAPL WAS WEE1 WHSC1 WHSC1L1 WNK1 WNK3 WNK4 WT1 WWTR1 XPO1 XRCC1 ZBTB16 ZBTB33 ZBTB7B ZC3H18 ZMYM2 ZMYM3 ZNF292 ZNF384 ZNF471 ZNF521 ZRSR2

RNA Targets and Gene Fusions (371 fusions)

ABI1 ABL1 ABL2 ACER1 ACSL6 ADD3 ADGRG7 AFF1 AFF3 AFF4 AHI1 ALK ANKRD28 AP2A2 ARHGAP20 ARHGAP26 ARHGEF12 ARHGEF17 ARNT ASXL1 ATF7IP ATIC AUTS2 BAALC BACH2 BAZ2A BCL10 BCL11A BCL11B BCL2 BCL2L1 BCL3 BCL5 BCL6 BCL7A BCL9 BCOR BCR BIRC3 BRD1 BRWD3 BTBD18 BTG1 C15orf65 CAPRIN1 CARS CASC5 CBFA2T3 CBFB CBL CCDC6 CCDC88C CCND1 CCND2 CCND3 CD274 CDK5RAP2 CDK6 CDX2 CEBPA CEBPB CEBPD CEBPE CEP170B CEP85L CHD6 CHIC2 CHST15 CIITA CLCA2 CLP1 CLTC CLTCL1 CNTRL CPSF6 CREBBP CRLF2 CUX1 DAB2IP DACH1 DACH2 DDX10 DDX6 DEK DMRT1 DTD1 DUSP22 EEFSEC EIF4A2 ELF4 ELL ELN EML1 ENAH EP300 EPOR EPS15 ERC1 ERG ERVK-6 ERVW-1 ETS1 ETV6 EWSR1 FAM46C FCGR2B FCRL4 FEN1 FGFR1 FGFR1OP FGFR1OP2 FGFR3 FIP1L1 FLT3 FNBP1 FOXO3 FOXO4 FOXP1 FRA7H FRYL FSTL3 FUS GAPDH GAS5 GAS6 GAS7 GATA1 GIT2 GLIS2 GMPS GOLGA4 GOLGA6A GOT1 GPHN GPR34 GRHPR HIP1 HIPK1 HIST1H4I HLF HMGA2 HOXA10 HOXA11 HOXA13 HOXA9 HOXC11 HOXC13 HOXD11 HRASLS5 HSP90AA1 HSP90AB1 ID4 IGF2BP1 IGH IGK IGL IKZF1 IL2 IL21R IL3 IQCG IRF4 IRS4 ITK JAK2 JAK3 KANK1 KAT6A KAT6B KDM5A KDSR KIAA1524 KIAA1549L KIF5B KMT2A KRAS KRT18P6 LASP1 LCK LCP1 LHX2 LHX4 LMBRD1 LMO1 LMO2 LNP1 LOC100289656 LPP LPXN LRMP LYL1 LYN MACROD1 MAF MAFB MALT1 MAML2 MAP3K9 MAPRE1 MBNL1 MBTD1 MDS2 MECOM MIR29A MKL1 MLF1 MLLT1 MLLT10 MLLT11 MLLT3 MLLT4 MLLT6 MN1 MNX1 MSI2 MSN MTCP1 MUC1 MYB MYC MYH11 MYH9 MYO18A NACA NAPA NBEAP1 NCKIPSD NCOA2 NCOA3 NCOR1 NDE1 NEBL NF1 NFKB2 NID2 NIN NIPBL NKX2-5 NOP2 NOTCH1 NPM1 NSD1 NTRK3 NUMA1 NUP214 NUP98 OLIG2 P2RY8 PAFAH1B2 PAK1 PAX5 PBX1 PCM1 PCSK7 PDCD1LG2 PDE4DIP PDGFB PDGFRA PDGFRB PER1 PHF21B PHF23 PICALM PIM1 PLAG1 PML POM121 POU2AF1 PPP1CB PRDM1 PRDM16 PRKAR1A PRKG2 PRRX1 PRRX2 PSIP1 PSMD2 PTPRR PVT1 RABEP1 RALGDS RANBP17 RANBP2 RAP1GDS1 RARA RBM15 RCSD1 RHOA RHOH RMI2 RNF213 RNF217-AS1 RPL22 RPN1 RUNX1 RUNX1T1 SARNP SART3 SEC31A SEPT2 SEPT5 SEPT6 SEPT9 SET SETBP1 SFPQ SH3D19 SH3GL1 SLCO1B3 SNHG5 SNX29 SORBS2 SPECC1 SPTBN1 SQSTM1 SRSF3 SSBP2 ST6GAL1 STAT5B STIL STRN SYK TAF15 TAL1 TAL2 TAOK1 TCF3 TCL1A TCL6 TCTA TET1 TFG TFPT TFRC THADA TLX1 TLX3 TMEM255B TNFRSF17 TOP1 TP53BP1 TP63 TPM3 TPM4 TRA TRAF1 TRB TRD TRIM24 TRIP11 TRPS1 TTL TYK2 USP16 USP42 WHSC1 WHSC1L1 XBP1 YPEL5 YTHDF2 ZBTB16 ZFP64 ZFPM2 ZFYVE19 ZMIZ1 ZMYM2 ZMYND11 ZNF384 ZNF521 ZNF687

MyMRD™

MyMRD™ - NGS Panel

Intended Use

The MyMRD is a RUO hotspot panel that detects all classes of variants identified in a precisely defined set of targets that commonly drive myeloid malignancies including AML, MPN and MDS. It can detect SNV, indels and translocations to the genomic basepair, giving unparalleled precision and detection of low level mutations in patients.

Testing with MyMRD allows for studying important mutations in known genes implicated in the causation, prognosis, and reoccurrence of myeloid disorders.

Summary and Explanation of the Test

Indexed whole genome libraries are hybridized with MyMRD probes targeting mutation hotspots in a total of 23 genes (A*SXL1 BRAF CALR CEBPA CSF3R DNMT3A FLT3 IDH1 IDH2 JAK2 KIT KRAS MPL NPM1 NRAS PTPN11 RUNX1 SF3B1 SRSF2 TP53 ZRSR2 CBFB-MYH11 KMT2A RUNX1-RUNX1T1*). In addition to targeting single nucleotide variants (SNVs) and indels in the first 21 genes, 5 structural variant breakpoints within the final 3 genes are also targeted. Coupling comprehensive gene coverage with enhanced depth of coverage, long read lengths, and the power of our robust MyInformatics™ annotation software and bioinformatics database, MyMRD confidently and reproducibly detects mutations with a mutant allele frequency of $5x10^{-3}$, while some mutations, such as *FLT3* ITDs, are detected at mutation allele frequencies as low at $1x10^{-3}$.

Background

Minimal residual disease (MRD) detection has proven to be useful in the clinical management of patients with leukemia and can facilitate the development of new therapies. Patients with myeloid neoplasms are typically divided into different prognostic groups based upon both cytogenetics and traditional molecular profiles[1]; however, this may not reflect the heterogeneity of disease[2] that can be exploited using MRD assessment.

Cancer heterogeneity poses several challenges to monitor MRD in patients. Additionally, patient sample is usually very limited, and therefore, serial testing is many times not a viable option. Thus, the development of a sensitive and reliable assay to detect several mutations within one sample represents a significant advancement in guiding treatment decisions. With the MyMRD assay, one sample is enough to characterize at least one driving mutation in 90%-95% of all AMLs.

Reference

1. Arber, D.A. et al. (2016). The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood*, 127(20), 2391-2405.
2. Sperling, A.S. et al. (2017). The genetics of myelodysplastic syndrome: from clonal hematopoiesis to secondary leukemia. Nature Reviews. *Cancer*, 17(1), 5–19

Specimen Requirements

DNA

- 5 mL of peripheral blood in Heparin, EDTA or ACD
- 3 mL of bone marrow in Heparin, EDTA or ACD
- 1 µg of purified, high quality genomic DNA

Turnaround Time

7-10 business days

Shipping Conditions

Ambient or Cool; Do not freeze

Storage Conditions

Room Temp up to 72 hours / 4 °C up to 7 days

MyMRD Gene List

Genes Targeted

SNV and Indel Targets in Genes (Exons) (21 genes)
ASXL1 BRAF CALR CEBPA CSF3R DNMT3A FLT3 IDH1 IDH2 JAK2 KIT KRAS MPL NPM1 NRAS PTPN11 RUNX1 SF3B1 SRSF2 TP53 ZRSR2

Structural Variants (Translocations and Partial Tandem Duplications in Intronic Structures) (5 targets)
CBFB-MYH11 KMT2A RUNX1-RUNX1T1

Warranty and Liability

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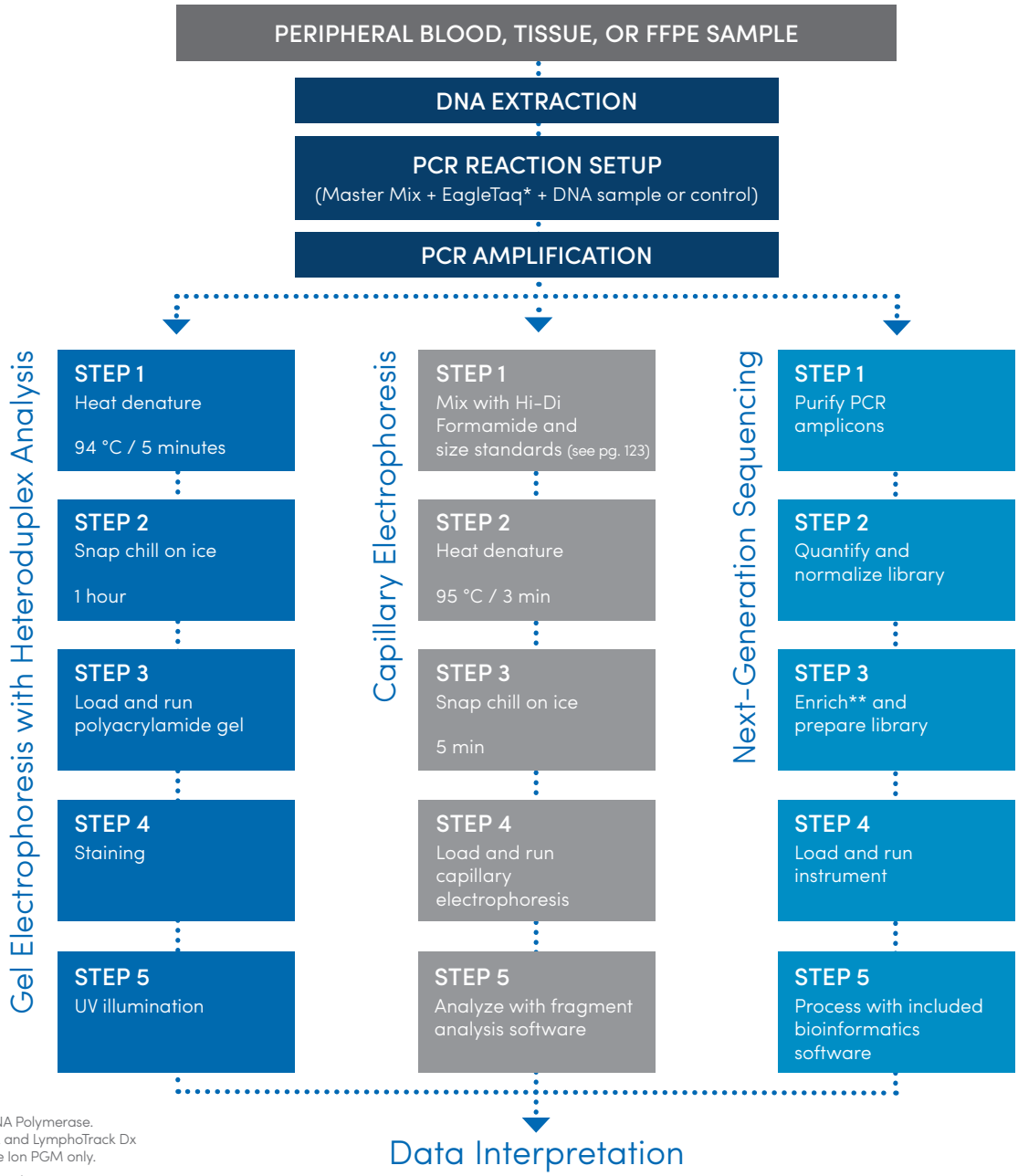
Reference

The Invivoscribe European Conformity marked *in vitro* diagnostics (CE-IVD) and Research Use Only (RUO) clonality assays detect clonal populations in just a few easy steps. These steps include PCR amplification of the immunoglobulin or T-cell receptor genes of interest, followed by detection with polyacrylamide gels, capillary electrophoresis, or next-generation sequencing using an Illumina® MiSeq® or Thermo Fisher Scientific® Ion PGM™ instrument. A flowchart illustrating this workflow is shown below.

Clonality Testing Workflow



*Or equivalent DNA Polymerase.
** For LymphoTrack and LymphoTrack Dx Assays run on the Ion PGM only.

CE-marked *in vitro* diagnostic products are not available for sale or use within North America.

LymphoTrack Dx and LymphoTrack Workflow Summary

Illumina® MiSeq®

STEP 1
Prepare DNA samples

STEP 2
Run PCR with a LymphoTrack Assay

STEP 3
Purify amplicons

STEP 4
Quantify and prepare library

STEP 5
Load onto MiSeq® flow cell and run

STEP 6
Process raw data with our included bioinformatics software

STEP 7
Analyze results

NOTICE: The LymphoTrack Dx Assays are *in vitro* diagnostic products and are not available for sale or use within North America.

*Image courtesy of Illumina, Inc.

LymphoTrack Dx and LymphoTrack Workflow Summary

Thermo Fisher Scientific® Ion PGM™

STEP 1
Prepare DNA samples

STEP 2
Run PCR with a LymphoTrack Assay

STEP 3
Purify amplicons

STEP 4
Quantify, prepare, and enrich library

STEP 5
Load onto Ion PGM™ chip and run

STEP 6
Process raw data with our included bioinformatics software

STEP 7
Analyze results

NOTICE: The LymphoTrack Dx Assays are *in vitro* diagnostic products and are not available for sale or use within North America.

*Image courtesy of Thermo Fisher Scientific

Next-Generation Sequencing Menu

Invivoscribe offers LymphoTrack and LymphoTrack Dx Assays for the analysis of B- and T-cell clonality, somatic hypermutation, and minimal residual disease studies**. Assays are designed for use on both industry standard next-generation sequencing (NGS) platforms: the Illumina® MiSeq® and Thermo Fisher Scientific® Ion PGM™ instruments.

Invivoscribe assays for the Illumina® MiSeq® platform offer the ability to analyze up to 22 samples and 2 controls per gene target and the multiplexing capabilities to generate a sequencing library that combines amplicons from different Invivoscribe LymphoTrack and LymphoTrack Dx Assays onto the same flow cell. Our included software then sorts and assigns the correct sequences to their corresponding sample.

Invivoscribe assays for the Ion PGM platform offer the ability to analyze up to 10 samples and 2 controls per gene target and the multiplexing capability to generate a sequencing library that combines amplicons from different Invivoscribe LymphoTrack and LymphoTrack Dx Assays onto the same sequencing chip, reducing per sample testing costs.

All LymphoTrack and LymphoTrack Dx Assays allow for fast and easy analysis and data visualization using the included bioinformatics software. The LymphoTrack software sorts and assigns the sequences to their corresponding sample and provides information such as the prevalence, gene segment usage, and the mutation rate (*IGH* Leader and *IGH* FR1 only). In addition, the Invivoscribe Minimal Residual Disease (MRD) Software allows for clonotype sequences to be tracked in subsequent samples for research applications.

The table below indicates which LymphoTrack (Research Use Only) and LymphoTrack Dx (CE-IVD Marked) Assays are currently available in 2018.

CE-Marked IVD Assays	MiSeq®	Ion PGM™
LymphoTrack® Dx *IGHV* Leader Somatic Hypermutation Assays	AVAILABLE	NOT OFFERED*
LymphoTrack® Dx *IGH* FR1 Assays	AVAILABLE	AVAILABLE
LymphoTrack® Dx *IGH* FR2 Assays	AVAILABLE	AVAILABLE
LymphoTrack® Dx *IGH* FR3 Assays	AVAILABLE	AVAILABLE
LymphoTrack® Dx *IGH* FR1/2/3 Assays	AVAILABLE	AVAILABLE
LymphoTrack® Dx *IGK* Assays	AVAILABLE	AVAILABLE
LymphoTrack® Dx *TRG* Assays	AVAILABLE	AVAILABLE
LymphoTrack® Dx *TRB* Assays	AVAILABLE	AVAILABLE
Research Use Only (RUO) Assays	**MiSeq®**	**Ion PGM™**
LymphoTrack® *IGHV* Somatic Hypermutation Assays	AVAILABLE	NOT OFFERED*
LymphoTrack® *IGH* FR1 Assays	AVAILABLE	AVAILABLE
LymphoTrack® *IGH* FR2 Assays	AVAILABLE	AVAILABLE
LymphoTrack® *IGH* FR3 Assays	AVAILABLE	AVAILABLE
LymphoTrack® *IGH* FR1/2/3 Assays	AVAILABLE	AVAILABLE
LymphoTrack® *IGK* Assays	AVAILABLE	AVAILABLE
LymphoTrack® *TRG* Assays	AVAILABLE	AVAILABLE
LymphoTrack® *TRB* Assay	AVAILABLE	AVAILABLE

* Due to the length of the amplicon and the available sequencing chemistries from Thermo Fisher Scientific, this assay is not available.

CE-marked assays are *in vitro* diagnostic products and are not available for sale or use within North America.
**Minimal residual disease (MRD) applications are currently for research use only.

Gel and Capillary Electrophoresis Menu

Invivoscribe offers assays that can be analyzed using two conventional methods of fragment analysis: gel electrophoresis or capillary electrophoresis.

Gel electrophoresis kits offer a comparatively easy and inexpensive solution for clonality, translocation, and mutational testing and are often the method of choice for laboratories new to using these methods and techniques. PCR products are analyzed using non-denaturing polyacrylamide gels (PAGE) and often require a heteroduplex step for resolution of generated amplicons.

Capillary electrophoresis kits are supplied with fluorescently labeled primers, allowing the resulting PCR products to be analyzed on Applied Biosystems (ABI) platforms (e.g. 3130, 3500). Fragment analysis by capillary electrophoresis offers the ability to detect fragments with a high level of accuracy and analytical sensitivity and allows for greater sample throughput compared to gel detection methods. In addition, capillary electrophoresis detection often facilitates a more objective interpretation of results than gel-based detection.

The table below summarizes which detection methods are available for our clonality and translocation assays either as Research Use Only or CE-marked IVDs.

CE-Marked IVD Assays	Gel	ABI
IdentiClone™ *IGH* + *IGK* B-Cell Clonality Assay	AVAILABLE	AVAILABLE
IdentiClone™ *IGH* Gene Clonality Assay	AVAILABLE	AVAILABLE
IdentiClone™ *IGK* Gene Clonality Assay	AVAILABLE	AVAILABLE
IdentiClone™ *IGL* Gene Clonality Assay	AVAILABLE	AVAILABLE
IdentiClone™ *TCRB* + *TCRG* T-Cell Clonality Assay	AVAILABLE	AVAILABLE
IdentiClone™ *TCRB* Gene Clonality Assay	AVAILABLE	AVAILABLE
IdentiClone™ T-Cell Receptor Gamma Gene Rearrangement Assay 2.0	NOT AVAILABLE	AVAILABLE
IdentiClone™ *TCRG* Gene Clonality Assay	AVAILABLE	AVAILABLE
IdentiClone™ *TCRD* Gene Clonality Assay	AVAILABLE	AVAILABLE
IdentiClone™ *BCL1*/JH Translocation Assay	AVAILABLE	NOT AVAILABLE
IdentiClone™ *BCL2*/JH Translocation Assay	AVAILABLE	NOT AVAILABLE
Research Use Only (RUO) Assays	**Gel**	**ABI**
IGH + *IGK* B-Cell Clonality Assay	AVAILABLE	AVAILABLE
IGH Gene Rearrangement Assay	AVAILABLE	AVAILABLE
IGH Gene Clonality Assay	AVAILABLE	AVAILABLE
IGH Somatic Hypermutation Assay v2.0	AVAILABLE	AVAILABLE
IGL Gene Clonality Assay	AVAILABLE	AVAILABLE
TCRB + *TCRG* T-Cell Clonality Assay	AVAILABLE	AVAILABLE
TCRB Gene Clonality Assay	AVAILABLE	AVAILABLE
T-Cell Receptor Gamma Gene Rearrangement Assay	AVAILABLE	AVAILABLE
T-Cell Receptor Gamma Gene Rearrangement Assay 2.0	NOT AVAILABLE	AVAILABLE
TCRG Gene Clonality Assay	AVAILABLE	AVAILABLE
TCRD Gene Clonality Assay	AVAILABLE	AVAILABLE
BCL1/JH Translocation Assay	AVAILABLE	NOT AVAILABLE
BCL2/JH Translocation Assay	AVAILABLE	NOT AVAILABLE
BCL2/JH t(14;18) Translocation Assay	AVAILABLE	NOT AVAILABLE
BCR/ABL t(9;22) Translocation Assay	AVAILABLE	AVAILABLE
PML/RARA t(15;17) Translocation Assay	AVAILABLE	AVAILABLE

CE-marked assays are *in vitro* diagnostic products and are not available for sale or use within North America.

Hematologic Malignancy Research and Testing Guide

	IGH (V_H - J_H)	*IGH* (D_H - J_H)	*IGK*	*IGL*	*IGHV* Somatic Hypermutation	*TRB*	*TRD*	*TRG*	*IGH-CCND1*	*IGH - BCL2*	*BCR-ABL1*	*PML-RARA*	*FLT3*	*NPM1*
Acute Myeloid Leukemia[7,10]													❍	❍
Acute Promyelocytic Leukemia (AML-M3)[1,5]												❍		
Chronic Myeloid Leukemia[9]											❍			
Acute Lymphoblastic Leukemia[4]	❍	❍	❍	◆		❍	❍	❍			❍			
Chronic Lymphocytic Leukemia[13,17]	❍	◆	❍	◆	❍	◆	◆	◆						
Multiple Myeloma[11,12]	◆													
T-Cell Large Granular Lymphocytic Leukemia[14,16]			◆	◆		❍	◆	❍						
T-Cell Prolymphocytic Leukemia[14,16]	◆	◆	◆	◆		❍	◆	❍						
Anaplastic Large-Cell Lymphoma[14,16]						❍	◆	❍						
Angioimmunoblastic T-Cell Lymphoma[14,16]	◆	◆	◆	◆		❍	◆	❍						
Diffuse Large B-Cell Lymphoma[3,8,14,15]	❍	◆	❍	◆		◆	◆	◆		❍				
Follicular Lymphoma[3,8,14,15]	❍	◆	❍	◆		◆	◆	◆		❍				
Mantle Cell Lymphoma[2,6,14,15]	❍	◆	❍	◆		◆	◆	◆	❍					
Marginal Zone Lymphoma[14,15]	❍	◆	❍	◆		◆	◆	◆						
Peripheral T-Cell Lymphoma[14,16]	◆	◆	◆			❍	◆	❍						
Small Lymphocytic Lymphoma[13,17]	❍	◆	❍	◆		◆	◆	◆						
Suspected B-Cell Proliferations[14]	❍	❍	❍	◆		◆	◆	◆						
Suspected T-Cell Proliferations[14]	◆	◆	◆	◆		❍	◆	❍						
Suspected Lymphoid Proliferations of Unknown Origin[14]	❍	❍	❍	◆		❍	◆	❍						

❍ Recommended Primary Test ◆ Recommended Secondary Test

To receive a complimentary copy of this guide as a wall chart, e-mail marketing@invivoscribe.com.

References

1. Kakizuka, A et al., Cell (1991) 66:675-684.
2. Rimokh, R et al., Blood (1994) 83:1871-1875.
3. Gribben, JG et al., Blood (1994) 83:3800-3807.
4. Radich, JP et al., Leukemia (1994) 8:1688-1695.
5. Gallagher, RE et al., Blood (1995) 86:1540-1547.
6. De Boer, CJ et al., Blood (1995) 86:2715-2723.
7. Mrózek K, Döhner H, and Bloomfield CD. Curr Opin Hematol. (2007) 14:106-114.
8. Rezuke, WN et al., Clinical Chemistry (1997) 43:1814-1823.
9. Deininger, MW et al., Blood (2000) 96:3343-56.
10. Schlenk RF et al., N Engl J Med. (2008) 358:1909-1918.
11. Gonzalez, D et al., Leukemia (2003) 17:1051-1057.
12. Gonzalez, D et al., Leukemia (2003) 17:1398-1403.
13. Ghia, P et al., Leukemia (2007) 21:1-3.
14. van Krieken, JH et al., Leukemia (2007) 21:201-6.
15. Evans, PAS et al., Leukemia (2007) 21:207-214.
16. Bruggemann, M et al., Leukemia (2007) 21:215-221.
17. Davi, F et al., Leukemia (2008) 22:212-214.
18. Döhner, H et al., Blood (2010) 115:453-474.
19. Huang, W et al., Blood (1993) 82:1264-1269.
20. Hughes, T et al., Blood (2006) 108:28-37.
21. Westbrook, CA et al., Blood (1992) 80:2983-2990.
22. Ralph, QM et al., Blood (1993) 82:202-206.

Common Technical Support Questions

1. **What sample types may be suitable for analysis with Invivoscribe Gel and Capillary assays?**

   We recommend high-quality DNA for clonality testing with our assays. This can be extracted from frozen or fresh tissue, peripheral blood, bone marrow, skin biopsies, etc.

2. **When should the recommended controls be run with our assays?**

   The no template, positive, and negative controls should be included in every run for each target, per the product insert or instructions for use.

3. **What is the purpose of the Specimen Control Size Ladder and Amplification Control master mix? What is the difference between these master mixes?**

   The Specimen Control Size Ladder and Amplification Control master mixes are used as troubleshooting tools that allow you to determine if the quality and quantity of your DNA sample is suitable for use with our assays. The Specimen Control Size Ladder amplifies DNA at approximately 100, 200, 300, 400, and 600 base pairs; whereas, the Amplification Control amplifies DNA at 235 bp.

4. **How should the master mix and controls be stored and thawed?**

   The master mixes should be stored at -65 to -85 °C and should be thawed at room temperature and vortexed prior to use. If you intend to use master mixes multiple times, we recommend aliquoting the master mixes to minimize the number of freeze/thaw cycles. For the *FLT3* CDx Mutation Assay: Opened vials of master mixes stored frozen may incur up to 4 freeze thaw cycles. Opened vials of controls are stored at 2°C – 8°C for one week. Alternatively, opened vials of controls stored frozen may incur up to 8 freeze thaw cycles.

5. **Where can more information about the primers used in our assays be found?**

   Most primer information is proprietary to Invivoscribe and cannot be disclosed. We can, however, tell you the target area for the primers in each master mix, if you contact our support team by emailing support@invivoscribe.com or by calling +1 858-224-6600.

6. **Which targets are recommended for the study of B-cell malignancies?**

   The EuroClonality/BIOMED-2 Group has shown that combined testing of *IGH* and *IGK* achieves a clinical sensitivity of 99%. If purchasing these assays separately is cost prohibitive, our *IGH* + *IGK* Gene Clonality Assay (does not include *IGH* Tubes D and E) may be a feasible alternative option (see Figure 2 and Table 1 in *Leukemia* (2007) 21, 201-206). We also offer next-generation sequencing LymphoTrack® Assays for *IGH* and *IGK* for use with MiSeq® or Ion PGM™ instruments. In addition, a high percentage of B-ALL patients have *TRG* rearrangements, which can be detected using our assays to detect *TRG* gene rearrangements.

7. **What are the differences between our *IGH* Gene Rearrangement Assays and the *IGH* Gene Clonality Assays?**

   The *IGH* Gene Rearrangement Assay was designed by Invivoscribe; whereas, the *IGH* Gene Clonality Assay was designed by the EuroClonality/BIOMED-2 Group. Both assays target the conserved IGH framework regions, Framework 1, Framework 2, and Framework 3. The *IGH* Gene Clonality Assay also targets incomplete D_H - J_H rearrangements. The *IGH* Gene Clonality Assay includes 33 reactions per master mix and the *IGH* Gene Rearrangement Assay includes 30 reactions per master mix.

8. **What do *IGH* Tubes D and E target do and why are they challenging to interpret?**

   Tubes D and E of our *IGH* Gene Clonality Assays target incomplete *IGH* D_H - J_H rearrangements. It is common to see known amplicons listed in the instructions for use in cases where a polyclonal background is absent (this is likely because these rearrangements are rare). Some of our customers are concerned by this, especially because there may be some samples that have robust germline amplification greater than the valid size range. We do not expect the germline amplification to outcompete true D_H - J_H rearrangements. PCR amplicons generated from germline templates are much larger than true D_H - J_H rearrangements. As a result, PCR products of germline amplifications are less robust when a specific target is present in samples.

9. **Why does the polyclonal control produce a peak around 148 bp when amplified with *IGK* Tube A – 6FAM?**

   The 148 bp peak is a result of the restricted repertoire of *IGK* and this peak commonly appears flanked by several smaller peaks on each side. It is still possible to have a true clonal rearrangement at this size in samples. If you suspect that this peak is clonal in one of your samples, we recommend following up with heteroduplex analysis. Alternatively, NGS-based LymphoTrack® and LymphoTrack® Dx Assays provide an easier interpretation for *IGK* and reduces the number of master mixes to just one reaction.

10. **What T-cell receptor kits would you recommend to detect T-cell clonal rearrangements?**

    Ideally, you should perform tests for *TRB*, *TRG*, and *TRD* to achieve the highest sensitivity. The EuroClonality/BIOMED-2 Group has shown that testing both *TRB* and *TRG* offers roughly the same sensitivity for the detection of T-cell malignancies as testing all three targets; however, they highly recommend testing all three assays in parallel to achieve optimal clinical sensitivity. *TRD* is especially useful in cases of suspected immature T-cell proliferations (see Figure 2 and Table 2 in *Leukemia* (2007) 21, 201-206). We also offer NGS kits for *TRG* for use with MiSeq® or Ion PGM™ instruments and for *TRB* for use with MiSeq®.

11. **What are the differences between the *TCRG* Gene Clonality Assay and the T-Cell Receptor Gamma Gene Rearrangement Assay 2.0?**

    The *TCRG* Gene Clonality Assay was designed by the EuroClonality/ BIOMED-2 Group and consists of two master mixes. For polyclonal populations, four Gaussian distributions are

generated. The T-Cell Receptor Gamma Gene Rearrangement Assay 2.0 was designed by Invivoscribe and targets all functional VH - JH rearrangements in a single master mix and produces smaller amplicons grouped under a single Gaussian distribution. This allows for easier interpretation and makes the assay more suitable for DNA extracted from FFPE tissue, which may consist of partially degraded DNA that would not amplify well with the larger valid size range of the *TCRG* Gene Clonality Assay.

12. What are the differences between the *IGH-BCL2* Translocation Assay and the *IGH-BCL2* t(14;18) Translocation Assay?

The *IGH-BCL2* Translocation Assay was designed by the EuroClonality/BIOMED-2 Group and is available as either a CE-IVD or research use only assay whereas; the *IGH-BCL2* t(14;18) Translocation Assay was designed by Invivoscribe and is only available for research use only. Both of these assays target MBR and Mcr translocations, but the *IGH-BCL2* Translocation Assay also targets the translocations at the 3' Mbr. The *IGH-BCL2* t(14;18) Translocation Assay was designed as a nested PCR allowing greater sensitivities (1 clonal cell per 10,000 normal cells) to be achieved. The limit of detection of the *IGH-BCL2* Translocation Assay is 1 clonal cell per 100 normal cells. Lastly, the *IGH-BCL2* Translocation Assay includes 33 reactions, whereas the *IGH-BCL2* t(14;18) Translocation Assay includes 30 reactions.

13. Do you offer quantitative chromosome translocation (e.g., *BCR-ABL1*) controls?

Our controls are validated for qualitative use, although our customers do successfully use them with quantitative assays. Unfortunately, we cannot guarantee their performance with any assay that was not designed by Invivoscribe.

14. Which capillary electrophoresis instruments are currently validated for use with our assay kits?

Currently the capillary electrophoresis instruments Invivoscribe has validated include: ABI 3100 and 3130 series for all capillary electrophoresis detection assays. The ABI 310 and 3500 instrument series have also been validated for the majority of our capillary electrophoresis detection assays. We are not able to support using instruments not listed as validated in the instructions for use of our CE-IVD assays.

15. What are the recommended settings for my ABI instrument?

Instruments should be calibrated with the DS-30 matrix standards (Dye set D) for the ABI 310, 3100, or 3130 instrument series. For the ABI 3500 sequencer series, we advise that you calibrate the instrument with DS-33 matrix standards. We also recommend using either POP-4 or POP-7 depending on which ABI instrument you are using. If your equipment supports POP-7, we recommend using this polymer as it can be utilized for both fragment analysis and sequencing; whereas, POP-4 can only be utilized for fragment analysis.

16. How should peaks outside the valid size range be interpreted when using assay kits?

You should not interpret peaks outside of the valid size range; although, in theory, it is possible to have a true rearrangement fall outside this region. If you are concerned about a suspect peak, you may sequence your product for confirmation. Please note that samples should always be interpreted within the context of all available clinical information.

17. Is cell-free DNA (cfDNA) a suitable sample type for Invivoscribe LymphoTrack® or LymphoTrack® Dx Assays?

The average size of cfDNA (~170 bps) makes it a suitable sample type to run with *IGH* FR3 master mixes. The use of cfDNA with *TRG* master mixes might be possible, but expected amplicon sizes generated with this assay are near the upper limits of the fragment lengths typically found with this sample type.

18. Is DNA extracted from FFPE tissue suitable to use with Invivoscribe LymphoTrack® or LymphoTrack® Dx Assays?

To ensure DNA from challenging specimens is of sufficient quality and quantity to generate a valid result, samples may be tested with the Specimen Control Size Ladder master mix.

19. On which instruments can I use the LymphoTrack® and LymphoTrack® Dx Assays?

We have different versions of our assays for the PGM™ and MiSeq® instruments (LymphoTrack *TRB* is currently available only on MiSeq®). No other DNA sequencers (e.g. 454) are currently supported. Assays for the Ion PGM™ and MiSeq® platforms differ slightly in terms of the total number of indices, etc., but both have similar benefits such as a one-step PCR reaction and included bioinformatics software.

20. How much DNA is needed for the LymphoTrack® and LymphoTrack® Dx Assays?

50 ng of high-quality genomic DNA is required for the Ion PGM™ and MiSeq® LymphoTrack and LymphoTrack Dx Assays for clonality and somatic hypermutation applications.

21. Can I use a different library quantification method or kit?

We recommend using the KAPA™ kit for MiSeq® assays and either the 2100 Bioanalyzer® or the LabChip® GX for the Ion PGM™ assays.

22. Will the LymphoTrack® or LymphoTrack® Dx analysis software work on my computer?

The software requires Microsoft Windows 7 (64-bit) and Excel 2007, 2010, or 2013 and will work with most desktop or laptop PCs. For specific requirements please refer to the software instructions for use.

23. Can I use the LymphoTrack® or LymphoTrack® Dx bioinformatics software with a different assay?

No, the software will only work with datasets obtained by our LymphoTrack and LymphoTrack Dx Assays.

24. What characters can I use when naming my samples and the file pathways? What types of files are accepted by the LymphoTrack® and LymphoTrack® Dx Software - MiSeq®?

Our software only recognizes file names and pathways that contain the following characters (A-Z, a-z, 0-9, . (dot), _ (underscore), - (hyphen)). In addition, spaces in the pathname for the data files or software (pathnames include file folders and file names) should be avoided. If the software encounters a character that is not listed above or extra spaces, an error

message may be generated. Furthermore, the software is only compatible with adaptor-trimmed fastq.gz files that are generated by the MiSeq® Reporter Software when the MiSeq® instrument is used. An example of the naming format that the MiSeq® Reporter uses: SampleName_S1_L001_R1_001.fastq.gz and SampleName_S1_L001_R2_001.fastq.gz.

25. Do the Invivoscribe MiSeq® indices correspond to the Illumina® indices?

The indices included in our MiSeq® master mixes follow Illumina®'s TruSeq LT nomenclature. For instance, *IGH* FR1 MiSeq® 01 corresponds to A001. Information for the other indices can be found in the instructions for use on how to set up the MiSeq® Sample Sheet to detect the appropriate indices.

26. Why am I getting a low percent passing filter and Q30 score?

Low Q30 and percent passing filter (%PF) scores could be an indication that the flow cell is overloaded. If this is suspected, verify your amplicon and library calculations and quantifications are correct. Low run metrics can also be attributed to many additional factors including poor quality DNA, contamination, flow cell or instrument issues, etc. Please refer to your Illumina MiSeq® user guides and contact Illumina® Support.

27. Why is the same V_H-J_H rearrangement combination and sequence shared by two groups of reads, one of which is several bases shorter than the other when looking at the Read Summary tab of the excel document created by the LymphoTrack® Visualization Tool?

Our software was designed to list every unique sequence separately in order for the customer to see all of the data and make their own determination on how to interpret it. The several base pair difference can be due to a number of factors including amplification errors and sequencing errors. It could also be a result of similarities between some of the primer sequences that were designed to ensure maximum coverage. We also include a Merged Read Summary report for your reference that combines sequences that only differ by 1 or 2 basepairs.

28. Do I need to perform an adapter ligation prior to sequencing my products?

Performing an adapter ligation is not needed. The primers included in our LymphoTrack and LymphoTrack Dx master mixes already include the appropriate index barcodes and adapter sequences. After PCR amplification, you will be able to proceed with amplicon purification, amplicon quantification, library pooling, and sequencing.

29. If the LymphoTrack® or LymphoTrack® Dx software generated an error, what information should I submit to Technical Support?

Please submit the *.txt Log file that should have been created by the software in the output folder, a screenshot of the sample directory, and the Lot Number of the software CD you are using to support@invivoscribe.com.

30. Are controls provided with the kits? Can you purchase additional controls? How are they supplied?

Each kit contains the necessary positive and negative controls required to perform the assay; additional controls may also be purchased separately. Single-tube DNA controls are provided as 100 µL aliquots of 200 µg/mL in 1/10 TE Buffer, 50 µL aliquots of 50 µg/mL in 1/10 TE Buffer, and 45 µL aliquots of 15 µg/mL in 1/10 TE Buffer. Single-tube RNA controls are provided as 100 µL aliquots of 400 µg/ml in RNAse free in glass distilled water.

31. What are the differences between dilution sets, sensitivity panels, and proficiency panels?

31a. RNA Dilution Sets
BCR/ABL b3a2 (Cat# 4-085-0210), *BCR/ABL* b2a2 (Cat# 4-085-0310), and *BCR/ABL* e1a2 (Cat# 4-085-0110) These sets contain six tubes: 100% negative control RNA and volume to volume (v/v) dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}) of the positive control RNA into the negative control RNA (IVS-0035). The undiluted concentration of the positive control RNA is 400 µg/mL and each tube contains 50 µL.

31b. RNA Sensitivity Panels
These panels consist of seven tubes: 100% positive control RNA and v/v dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}) of the positive control RNA into the negative control RNA (IVS-0035). The undiluted concentration of the positive control RNA is 400 µg/mL and each tube contains 100 µL.

31c. DNA Sensitivity Panels
Consist of six tubes: 100% clonal positive DNA and v/v dilutions of the positive clonal DNA into negative polyclonal DNA (IVS-0000) to make 30%, 20%, 10%, 5%, and 1% dilutions. The undiluted DNA has a concentration of 200 µg/mL and each tube contains 100 µL.

31d. RNA Proficiency Panel
The proficiency panel for *BCR-aBL1* t(9;22) comes with ten tubes: (See table below)

RNA Proficiency Panel

Qty	Description	Chromosome Translocation
1	IVS-0003 Clonal Control RNA 10^{-2} IVS-0003 Clonal Control RNA 10^{-4} IVS-0003 Clonal Control RNA	*BCR-ABL1* p210 e13a2 (b2a2)
1	IVS-0011 Clonal Control RNA 10^{-2} IVS-0011 Clonal Control RNA 10^{-4} IVS-0011 Clonal Control RNA	*BCR-ABL1* p210 e14a2 (b3a2)
1	IVS-0032 Clonal Control RNA 10^{-2} IVS-0032 Clonal Control RNA 10^{-4} IVS-0032 Clonal Control RNA	*BCR-ABL1* p190 e1a2
1	IVS-0035 Clonal Control RNA	*BCR-ABL1* Negative

Recent Poster Abstracts

Genetic Heterogeneity and Stratification of AML Samples with *NPM1* Mutation Detected by the MyAML® NGS Test
Poster presented at: Association for Molecular Pathology 2017 Annual Meeting. 2017 November 16-18, Salt Lake City, UT, USA.

Clonality Detection Using Next-Generation Sequencing and Capillary Electrophoresis Methods in Suspect Lymphoproliferative Samples
Poster presented at: Association for Molecular Pathology 2017 Annual Meeting. 2017 November 16-18, Salt Lake City, UT, USA.

Validation of the LeukoStrat® CDx *FLT3* Mutation Assay: Used to Detect both Internal Tandem Duplication (ITD) and Tyrosine Kinase Domain (TKD) Mutations and Response to Midostaurin in 1058 Patients with AML
Poster presented at: Association for Molecular Pathology 2017 Annual Meeting. 2017 November 16-18, Salt Lake City, UT, USA.

Multiple Highly Concordant Assays Facilitate Analyses of Clinical Samples at Different Scales and Sensitivities
Poster presented at: Association for Molecular Pathology 2017 Annual Meeting. 2017 November 16-18, Salt Lake City, UT, USA.

Detection of Clonal *TRG* and *TRB* Gene Rearrangements Using Next-Generation Sequencing
Poster presented at: Association for Molecular Pathology 2017 Annual Meeting. 2017 November 16-18, Salt Lake City, UT, USA.

Analysis and Characterization of Hematologic Cancers Using a Comprehensive NGS Panel Comprised of DNA and RNA Baits Targeting 704 Genes
Poster presented at: 22nd Congress of the European Hematology Association. 2017 June 22-25. Madrid, Spain.

Next Generation Sequencing Targeted Panel for Minimal Residual Disease Monitoring in Acute Myeloid Leukemia
Poster presented at: 22nd Congress of the European Hematology Association. 2017 June 22-25. Madrid, Spain.

Detection of Clonality in Clinical Specimens from Suspected B-Cell Malignancies Using Comprehensive *IGH* Lymphotrack® MiSeq® and PGM® Assays
Poster presented at: 22nd Congress of the European Hematology Association. 2017 June 22-25. Madrid, Spain.

Small Customizable NGS Based Target Capture Panels Detect Variants in Clinical Specimens at Frequencies as Low as 0.5%
Poster presented at: 22nd Congress of the European Hematology Association. 2017 June 22-25. Madrid, Spain.

Detecting B-Cell Clonality in Clinical Samples using a Comprehensive NGS LymphoTrack Dx® *IGH* FR1/2/3 Assay
Poster presented at: Association for Molecular Pathology Global 2017 Meeting. 2017 April 3-5. Berlin, Germany.

Assessment of Minimal Residual Disease in Patients with Acute Myeloid Leukemia by Monitoring *FLT3* and *NPM1* Mutations
Poster presented at: Association for Molecular Pathology Global 2017 Meeting. 2017 April 3-5. Berlin, Germany.

A Precision Medicine Approach Incorporating Both Molecular and In Vitro Functional Data to Treat Patients with Relapsed/Refractory Acute Myeloid Leukemia
Poster Presented at: 58th Annual American Society for Hematology Meeting. 2016 Dec. 3-6. San Diego, CA, USA.

Precision Medicine Assays Detect Novel Targetable *FLT3* Fusions Amenable to Therapeutic Intervention in a Patient with Refractory Acute Myeloid Leukemia
Poster Presented at: 58th Annual American Society for Hematology Meeting. 2016 Dec. 3-6. San Diego, CA, USA.

Next-Generation Sequencing of *FLT3/ITD* for Minimal Residual Disease Monitoring in Leukemia Patients
Poster Presented at: Association for Molecular Pathology 2016 Annual Meeting. November 10-12, 2016 at the Charlotte Convention Center in Charlotte, NC, USA.

Next-Generation Sequencing of *NPM1* for Minimal Residual Disease Monitoring in Leukemia Patients
Poster Presented at: Association for Molecular Pathology 2016 Annual Meeting. November 10-12, 2016 at the Charlotte Convention Center in Charlotte, NC, USA.

Detection of Clonal Immunoglobulin and T-Cell Receptor Gene Rearrangements in Acute Myeloid Leukemia
Poster Presented at: Association for Molecular Pathology 2016 Annual Meeting. November 10-12, 2016 at the Charlotte Convention Center in Charlotte, NC, USA.

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Development of a Comprehensive *IGH* NGS Assay for Detecting Suspected B-Cell Clonality
Poster Presented at: Association for Molecular Pathology 2016 Annual Meeting. November 10-12, 2016 at the Charlotte Convention Center in Charlotte, NC, USA.

Detection of Minimal Residual Disease in *FLT3/ITD* AML
Poster presented at: American Society of Clinical Oncology 2016 Annual Meeting. June 3-7, 2016 at McCormick Place in Chicago, Illinois, USA.

Development of LymphoTrack® Bioinformatics Methods: Clonality Testing, Somatic Hypermutation, and Minimal Residual Disease
Poster Presented at: Association for Molecular Pathology 2015 Annual Meeting. 2015 Nov 4-8. Austin, TX, USA.

Detection of Clonal *TRG* Gene Rearrangements Shows Improved Sensitivity and Positive Predictive Value Compared to Fragment Analysis Using BIOMED-2 Primers and Capillary Electrophoresis
Poster Presented at: Association for Molecular Pathology 2015 Annual Meeting. 2015 Nov 4-8. Austin, TX, USA.

Development of an NGS Assay for *IGK* that can be Combined with *IGH* for Identifying Clonal Populations in Lymphoid Malignancies
Poster Presented at: 20th Congress of the European Hematology Association. 2015 June 11-14. Vienna, Austria.

Clinical Assessment of Chronic Lymphocytic Leukemia (CLL) samples for Somatic Hypermutation Status by Next-Generation Sequencing and Sanger Sequencing
Poster Presented at: 20th Congress of the European Hematology Association. 2015 June 11-14. Vienna, Austria.

Somatic Hypermutation and V-J Gene Usage for CLL Prognosis: Evaluating Data from MiSeq® NGS vs. PCR-Sanger Sequencing Approaches
Poster Presented at: 56th Annual American Society for Hematology Meeting. 2014 Dec. 6-9. San Francisco, CA, USA.

International Validation of a Harmonized NGS PGM™ Assay at Clinical Labs in the US and the EU
Poster Presented at: Association for Molecular Pathology 2014 Annual Meeting. 2014 Nov 13-15. National Harbor, MD, USA.

Performance of an *IGH* Somatic Hypermutation Assay with Associated LymphoTrack® Bioinformatics Developed for the MiSeq® NGS Platform
Poster Presented at: 19th Congress of the European Hematology Association. 2014 June 12-15. Milan, Italy.

Concordance in *IGH* & *TRG* Clonality Testing: Comparison of Data Generated Using the MiSeq® & PGM™ Platforms
Poster Presented at: 19th Congress of the European Hematology Association. 2014 June 12-15. Milan, Italy.

Combined *TRG* and *IGH* Clonality Testing on the PGM™ Using LymphoTrack® Reagents & Bioinformatics
Poster Presented at: 19th Congress of the European Hematology Association. 2014 June 12-15. Milan, Italy.

Identifying and Monitoring *IGH* Clonality Using Massively Parallel Sequencing and Associated Bioinformatics
Poster Presented at: Association for Molecular Pathology 2013 Annual Meeting. 2013 Nov. 15-16. Phoenix, AZ, USA.

Detecting Minimal Residual Disease Using a Massively Parallel Sequencing *TCRG* Assay
Poster Presented at: Association for Molecular Pathology 2013 Annual Meeting. 2013 Nov. 15-16. Phoenix, AZ, USA.

Identifying and Monitoring *TCRG* Clonality Using Massively Parallel Sequencing and Associated Bioinformatics
Poster Presented at: 18th Congress of the European Hematology Association. 2013 June 13-16. Stockholm, Sweden.

Warranty and Liability

Invivoscribe Technologies, Inc. (Invivoscribe®) is committed to providing the highest-quality products. Invivoscribe® warrants that the products meet or exceed the performance standards described in the Instructions For Use, as to products with such an insert. If a product is covered by product specifications and does not perform as specified, our policy is to replace the product or credit the full purchase price. No other warranties of any kind, expressed or implied, are provided by Invivoscribe®. Invivoscribe® liability shall not exceed the purchase price of the product. Invivoscribe shall have no liability for direct, indirect, consequential, or incidental damages arising from the use, results of use, or inability to use its products; product efficacy under purchaser controlled conditions in purchaser's laboratory must be established and continually monitored through purchaser defined and controlled processes including but not limited to testing of positive, negative, and blank controls every time a sample is tested. Ordering, acceptance, and use of product constitutes purchaser acceptance of sole responsibility for assuring product efficacy and purchaser agreement to the limitation of liability set forth in this paragraph.

Product List by Catalog Number

Research Use Only Assays
Capillary & Gel Fragment Analysis

1-100-0010 *IGH* + *IGK* B-Cell Clonality Assay – Gel Detection
1-100-0020 *IGH* + *IGK* B-Cell Clonality Assay MegaKit – Gel Detection
1-100-0031 *IGH* + *IGK* B-Cell Clonality Assay – ABI Fluorescence Detection
1-100-0041 *IGH* + *IGK* B-Cell Clonality Assay MegaKit – ABI Fluorescence Detection
1-101-0010 *IGH* Gene Rearrangement Assay – Gel Detection
1-101-0020 *IGH* Gene Clonality Assay – Gel Detection
1-101-0030 *IGH* Gene Rearrangement Assay MegaKit – Gel Detection
1-101-0040 *IGH* Gene Clonality Assay MegaKit – Gel Detection
1-101-0051 *IGH* Gene Rearrangement Assay – ABI Fluorescence Detection
1-101-0061 *IGH* Gene Clonality Assay – ABI Fluorescence Detection
1-101-0071 *IGH* Gene Rearrangement Assay MegaKit – ABI Fluorescence Detection
1-101-0081 *IGH* Gene Clonality Assay MegaKit – ABI Fluorescence Detection
1-102-0020 *IGK* Gene Clonality Assay – Gel Detection
1-102-0021 *IGK* Gene Clonality Assay – ABI Fluorescence Detection
1-102-0030 *IGK* Gene Clonality Assay MegaKit – Gel Detection
1-102-0031 *IGK* Gene Clonality Assay MegaKit – ABI Fluorescence Detection
1-103-0010 *IGL* Gene Clonality Assay – Gel Detection
1-103-0011 *IGL* Gene Clonality Assay – ABI Fluorescence Detection
1-103-0020 *IGL* Gene Clonality Assay MegaKit – Gel Detection
1-103-0021 *IGL* Gene Clonality Assay MegaKit – ABI Fluorescence Detection
1-200-0010 *TCRB* + *TCRG* T-Cell Clonality Assay – Gel Detection
1-200-0011 *TCRB* + *TCRG* T-Cell Clonality Assay – ABI Fluorescence Detection
1-200-0020 *TCRB* + *TCRG* T-Cell Clonality Assay MegaKit – Gel Detection
1-200-0021 *TCRB* + *TCRG* T-Cell Clonality Assay MegaKit – ABI Fluorescence Detection
1-205-0010 *TCRB* Gene Clonality Assay – Gel Detection
1-205-0011 *TCRB* Gene Clonality Assay – ABI Fluorescence Detection
1-205-0020 *TCRB* Gene Clonality Assay MegaKit – Gel Detection
1-205-0021 *TCRB* Gene Clonality Assay MegaKit – ABI Fluorescence Detection
1-206-0010 *TCRD* Gene Clonality Assay – Gel Detection
1-206-0011 *TCRD* Gene Clonality Assay – ABI Fluorescence Detection
1-206-0020 *TCRD* Gene Clonality Assay MegaKit – Gel Detection
1-206-0021 *TCRD* Gene Clonality Assay MegaKit – ABI Fluorescence Detection
1-207-0010 T-Cell Receptor Gamma Gene Rearrangement Assay – Gel Detection
1-207-0020 *TCRG* Gene Clonality Assay – Gel Detection
1-207-0021 *TCRG* Gene Clonality Assay – ABI Fluorescence Detection
1-207-0030 T-Cell Receptor Gamma Gene Rearrangement Assay MegaKit – Gel Detection
1-207-0040 *TCRG* Gene Clonality Assay MegaKit – Gel Detection
1-207-0041 *TCRG* Gene Clonality Assay MegaKit – ABI Fluorescence Detection
1-207-0051 T-Cell Receptor Gamma Gene Rearrangement Assay – ABI Fluorescence Detection
1-207-0071 T-Cell Receptor Gamma Gene Rearrangement Assay MegaKit – ABI Fluorescence Detection
1-207-0101 T-Cell Receptor Gamma Gene Rearrangement Assay 2.0 – ABI Fluorescence Detection
1-207-0111 T-Cell Receptor Gamma Gene Rearrangement Assay 2.0 MegaKit – ABI Fluorescence Detection
1-308-0010 *BCL1/J*H Translocation Assay – Gel Detection
1-308-0020 *BCL1/J*H Translocation Assay MegaKit – Gel Detection
1-309-0010 *BCL2/J*H t(14;18) Translocation Assay – Gel Detection
1-309-0020 *BCL2/J*H Translocation Assay – Gel Detection
1-309-0030 *BCL2/J*H t(14;18) Translocation Assay MegaKit – Gel Detection
1-309-0040 *BCL2/J*H Translocation Assay MegaKit – Gel Detection
1-310-0010 *BCR/ABL* t(9;22) Translocation Assay – Gel Detection
1-310-0020 *BCR/ABL* t(9;22) Translocation Assay MegaKit – Gel Detection
1-310-0031 *BCR/ABL* t(9;22) Translocation Assay – ABI Fluorescence Detection
1-310-0041 *BCR/ABL* t(9;22) Translocation Assay MegaKit – ABI Fluorescence Detection
1-311-0010 *PML/RARa* t(15;17) Translocation Assay – Gel Detection
1-311-0011 *PML/RARa* t(15;17) Translocation Assay – ABI Fluorescence Detection
1-311-0020 *PML/RARa* t(15;17) Translocation Assay MegaKit – Gel Detection
1-311-0021 *PML/RARa* t(15;17) Translocation Assay MegaKit – ABI Fluorescence Detection
1-412-0010 *FLT3* Mutation Assay - Gel Detection
1-412-0020 *FLT3* Mutation Assay MegaKit - Gel Detection
1-412-0031 *FLT3* Mutation Assay - ABI Fluorescence Detection
1-412-0041 *FLT3* Mutation Assay MegaKit - ABI Fluorescence Detection

Master Mixes

2-096-0010 Amplification Control Master Mix – Unlabeled
2-096-0011 Amplification Control Master Mix – 6FAM
2-096-0020 Specimen Control Size Ladder – Unlabeled
2-096-0021 Specimen Control Size Ladder – 6FAM
2-101-0010 *IGH* Tube A – Unlabeled
2-101-0011 *IGH* Tube A – 6FAM
2-101-0020 *IGH* Tube B – Unlabeled
2-101-0030 *IGH* Tube C – Unlabeled
2-101-0031 *IGH* Tube C – HEX
2-101-0040 *IGH* Tube D – Unlabeled
2-101-0041 *IGH* Tube D – HEX
2-101-0050 *IGH* Tube E – Unlabeled
2-101-0051 *IGH* Tube E – 6FAM
2-101-0060 *IGH* Framework 1 (FR1) – Unlabeled
2-101-0061 *IGH* Framework 1 (FR1) – 6FAM
2-101-0070 *IGH* Framework 2 (FR2) – Unlabeled
2-101-0080 *IGH* Framework 3 (FR3) – Unlabeled
2-101-0081 *IGH* Framework 3 (FR3) – HEX
2-101-0091 *IGH* Framework 2 (FR2) – 6FAM
2-101-0101 *IGH* Tube B – 6FAM
2-101-0170 Hypermutation Mix 1 v2.0 – Unlabeled
2-101-0171 Hypermutation Mix 1 v2.0 – 6FAM
2-101-0180 Hypermutation Mix 2 v2.0 – Unlabeled
2-101-0181 Hypermutation Mix 2 v2.0 – 6FAM
2-102-0010 *IGK* Tube A – Unlabeled
2-102-0011 *IGK* Tube A – 6FAM
2-102-0020 *IGK* Tube B – Unlabeled
2-102-0021 *IGK* Tube B – 6FAM
2-103-0010 *IGL* Tube – Unlabeled
2-103-0011 *IGL* Tube – 6FAM
2-205-0010 *TCRB* Tube A – Unlabeled
2-205-0011 *TCRB* Tube A – 6FAM & HEX
2-205-0020 *TCRB* Tube B – Unlabeled
2-205-0021 *TCRB* Tube B – 6FAM
2-205-0030 *TCRB* Tube C – Unlabeled
2-205-0031 *TCRB* Tube C – 6FAM & HEX
2-206-0010 *TCRD* Tube – Unlabeled
2-206-0011 *TCRD* Tube – 6FAM & HEX
2-207-0010 T-Cell Receptor Gamma Mix 1 – Unlabeled
2-207-0020 T-Cell Receptor Gamma Mix 2 – Unlabeled
2-207-0021 T-Cell Receptor Gamma Mix 2 – HEX
2-207-0030 *TCRG* Tube A – Unlabeled
2-207-0031 *TCRG* Tube A – 6FAM & HEX
2-207-0040 *TCRG* Tube B – Unlabeled
2-207-0041 *TCRG* Tube B – 6FAM & HEX
2-207-0071 T-Cell Receptor Gamma Mix 1 – 6FAM
2-207-0091 *TCRG* – 6FAM
2-308-0010 *BCL1/J*H Tube – Unlabeled
2-309-0010 *BCL2/J*H t(14;18) (Mbr) Mix 1b – Unlabeled
2-309-0020 *BCL2/J*H t(14;18) (mcr) Mix 2b – Unlabeled
2-309-0030 *BCL2/J*H t(14;18) (Mbr) Mix 1a – Unlabeled
2-309-0040 *BCL2/J*H t(14;18) (mcr) Mix 2a – Unlabeled
2-309-0050 *BCL2/J*H Tube A – Unlabeled
2-309-0060 *BCL2/J*H Tube B – Unlabeled
2-309-0070 *BCL2/J*H Tube C – Unlabeled
2-310-0010 *BCR/ABL* t(9;22) Mix 1a – Unlabeled
2-310-0020 *BCR/ABL* t(9;22) Mix 2a – Unlabeled
2-310-0030 *BCR/ABL* t(9;22) Mix 3a – Unlabeled
2-310-0040 *BCR/ABL* t(9;22) Mix 1b – Unlabeled
2-310-0041 *BCR/ABL* t(9;22) Mix 1b – HEX
2-310-0050 *BCR/ABL* t(9;22) Mix 2b – Unlabeled
2-310-0051 *BCR/ABL* t(9;22) Mix 2b – HEX
2-310-0060 *BCR/ABL* t(9;22) Mix 2c – Unlabeled
2-310-0061 *BCR/ABL* t(9;22) Mix 2c – HEX
2-310-0070 *BCR/ABL* t(9;22) Mix 3b – Unlabeled
2-310-0071 *BCR/ABL* t(9;22) Mix 3b – 6FAM
2-310-0080 *BCR/ABL* t(9;22) Mix 3c – Unlabeled
2-310-0081 *BCR/ABL* t(9;22) Mix 3c – 6FAM
2-310-0090 *BCR/ABL* t(9;22) Mix 3d – Unlabeled
2-310-0101 *BCR/ABL* t(9;22) Mix 3d – 6FAM
2-311-0011 *PML/RARa* t(15;17) Mix 1 – HEX
2-311-0031 *PML/RARa* t(15;17) Mix 2b – HEX
2-311-0041 *PML/RARa* t(15;17) Mix 2c – HEX

BCR/ABL RNA Dilution Sets

4-085-0110 *BCR/ABL* e1a2 RNA Dilution Set
4-085-0210 *BCR/ABL* b3a2 RNA Dilution Set
4-085-0310 *BCR/ABL* b2a2 RNA Dilution Set

DNA Sensitivity Panels

4-086-0040 Sensitivity Panel – IVS-0004 Clonal Control DNA
4-086-0070 Sensitivity Panel – IVS-0007 Clonal Control DNA
4-086-0090 Sensitivity Panel – IVS-0009 Clonal Control DNA
4-086-0100 Sensitivity Panel – IVS-0010 Clonal Control DNA
4-086-0190 Sensitivity Panel – IVS-0019 Clonal Control DNA
4-086-0210 Sensitivity Panel – IVS-0021 Clonal Control DNA
4-086-0300 Sensitivity Panel – IVS-0030 Clonal Control DNA

RNA Sensitivity Panels

4-087-0030 Sensitivity Panel – IVS-0003 Clonal Control RNA
4-087-0110 Sensitivity Panel – IVS-0011 Clonal Control RNA
4-087-0150 Sensitivity Panel – IVS-0015 Clonal Control RNA
4-087-0200 Sensitivity Panel – IVS-0020 Clonal Control RNA
4-087-0320 Sensitivity Panel – IVS-0032 Clonal Control RNA

Cell Line DNA Controls

4-088-0008 *IGH* SHM Positive Control DNA
4-088-0010 IVS-0001 Clonal Control DNA
4-088-0190 IVS-0004 Clonal Control DNA
4-088-0210 20% IVS-0004 Clonal Control DNA
4-088-0220 10% IVS-0004 Clonal Control DNA
4-088-0230 5% IVS-0004 Clonal Control DNA
4-088-0370 IVS-0007 Clonal Control DNA
4-088-0390 20% IVS-0007 Clonal Control DNA
4-088-0400 10% IVS-0007 Clonal Control DNA
4-088-0410 5% IVS-0007 Clonal Control DNA
4-088-0420 1% IVS-0007 Clonal Control DNA
4-088-0430 IVS-0008 Clonal Control DNA
4-088-0470 5% IVS-0008 Clonal Control DNA
4-088-0480 1% IVS-0008 Clonal Control DNA
4-088-0490 IVS-0009 Clonal Control DNA
4-088-0500 30% IVS-0009 Clonal Control DNA
4-088-0510 20% IVS-0009 Clonal Control DNA
4-088-0520 10% IVS-0009 Clonal Control DNA
4-088-0530 5% IVS-0009 Clonal Control DNA
4-088-0540 1% IVS-0009 Clonal Control DNA
4-088-0550 IVS-0010 Clonal Control DNA
4-088-0560 30% IVS-0010 Clonal Control DNA
4-088-0580 10% IVS-0010 Clonal Control DNA
4-088-0590 5% IVS-0010 Clonal Control DNA
4-088-0730 IVS-0013 Clonal Control DNA
4-088-1090 IVS-0019 Clonal Control DNA
4-088-1100 30% IVS-0019 Clonal Control DNA
4-088-1110 20% IVS-0019 Clonal Control DNA
4-088-1120 10% IVS-0019 Clonal Control DNA
4-088-1130 5% IVS-0019 Clonal Control DNA
4-088-1140 1% IVS-0019 Clonal Control DNA
4-088-1210 IVS-0021 Clonal Control DNA
4-088-1220 30% IVS-0021 Clonal Control DNA
4-088-1230 20% IVS-0021 Clonal Control DNA
4-088-1240 10% IVS-0021 Clonal Control DNA
4-088-1250 5% IVS-0021 Clonal Control DNA
4-088-1260 1% IVS-0021 Clonal Control DNA
4-088-1390 IVS-0024 Clonal Control DNA
4-088-1430 5% IVS-0024 Clonal Control DNA
4-088-1690 IVS-0029 Clonal Control DNA
4-088-1700 30% IVS-0029 Clonal Control DNA
4-088-1730 5% IVS-0029 Clonal Control DNA
4-088-1750 IVS-0030 Clonal Control DNA
4-088-1760 30% IVS-0030 Clonal Control DNA
4-088-1770 20% IVS-0030 Clonal Control DNA
4-088-1780 10% IVS-0030 Clonal Control DNA
4-088-1790 5% IVS-0030 Clonal Control DNA
4-088-1800 1% IVS-0030 Clonal Control DNA
4-088-1810 IVS-0031 Clonal Control DNA
4-088-1840 10% IVS-0031 Clonal Control DNA
4-088-1860 1% IVS-0031 Clonal Control DNA

Cell Line RNA Controls

4-089-0100 IVS-0002 Clonal Control RNA
4-089-0190 IVS-0003 Clonal Control RNA
4-089-0200 10^{-1} IVS-0003 Clonal Control RNA
4-089-0210 10^{-2} IVS-0003 Clonal Control RNA
4-089-0220 10^{-3} IVS-0003 Clonal Control RNA
4-089-0230 10^{-4} IVS-0003 Clonal Control RNA
4-089-0240 10^{-5} IVS-0003 Clonal Control RNA
4-089-0250 10^{-6} IVS-0003 Clonal Control RNA
4-089-0910 IVS-0011 Clonal Control RNA
4-089-0920 10^{-1} IVS-0011 Clonal Control RNA
4-089-0930 10^{-2} IVS-0011 Clonal Control RNA
4-089-0940 10^{-3} IVS-0011 Clonal Control RNA
4-089-0950 10^{-4} IVS-0011 Clonal Control RNA
4-089-0960 10^{-5} IVS-0011 Clonal Control RNA
4-089-1270 IVS-0015 Clonal Control RNA
4-089-1720 IVS-0020 Clonal Control RNA
4-089-1730 10^{-1} IVS-0020 Clonal Control RNA
4-089-1740 10^{-2} IVS-0020 Clonal Control RNA
4-089-1750 10^{-3} IVS-0020 Clonal Control RNA
4-089-1760 10^{-4} IVS-0020 Clonal Control RNA
4-089-2800 IVS-0032 Clonal Control RNA
4-089-2810 10^{-1} IVS-0032 Clonal Control RNA
4-089-2820 10^{-2} IVS-0032 Clonal Control RNA
4-089-2830 10^{-3} IVS-0032 Clonal Control RNA
4-089-2840 10^{-4} IVS-0032 Clonal Control RNA
4-089-2850 10^{-5} IVS-0032 Clonal Control RNA
4-089-2860 10^{-6} IVS-0032 Clonal Control RNA
4-089-3070 IVS-0035 Clonal Control RNA

Plasmid DNA Control

4-090-0070 IVS-P002 Clonal Control DNA

Tissue DNA Control

4-092-0010 IVS-0000 Polyclonal Control DNA

RNA Proficiency Panel

4-310-0100 Proficiency Panel for *BCR/ABL* t(9;22) Translocations

Somatic Hypermutation Sanger Sequencing Assays

5-101-0030 *IGH* Somatic Hypermutation Assay v2.0 – Gel Detection
5-101-0031 *IGH* Somatic Hypermutation Assay v2.0 – ABI Fluorescence Detection
5-101-0040 *IGH* Somatic Hypermutation Assay v2.0 MegaKit – Gel Detection
5-101-0041 *IGH* Somatic Hypermutation Assay v2.0 MegaKit – ABI Fluorescence

ABI Reagents

6-098-0051 HI-Deionized Formamide with ROX Size Standard (ABI 310)
6-098-0061 HI-Deionized Formamide with ROX Size Standard (ABI 3100)

Next-Generation Sequencing CE-IVD LymphoTrack® Dx Assays

9-121-0059 LymphoTrack® Dx *IGHV* Leader Somatic Hypermutation Assay Kit A – MiSeq®
9-121-0069 LymphoTrack® Dx *IGHV* Leader Somatic Hypermutation Assay Panel – MiSeq®
9-121-0129 LymphoTrack® Dx *IGH* FR1/2/3 Assay Kit A – MiSeq®
9-121-0139 LymphoTrack® Dx *IGH* FR1/2/3 Assay Panel – MiSeq®
9-121-0009 LymphoTrack® Dx *IGH* FR1 Assay Kit A – MiSeq®
9-121-0039 LymphoTrack® Dx *IGH* FR1 Assay Panel – MiSeq®
9-121-0089 LymphoTrack® Dx *IGH* FR2 Assay Kit A – MiSeq®
9-121-0099 LymphoTrack® Dx *IGH* FR2 Assay Panel – MiSeq®
9-121-0109 LymphoTrack® Dx *IGH* FR3 Assay Kit A – MiSeq®
9-121-0119 LymphoTrack® Dx *IGH* FR3 Assay Panel – MiSeq®
9-121-0057 LymphoTrack® Dx *IGH* FR1/2/3 Assay – PGM™
9-121-0007 LymphoTrack® Dx *IGH* FR1 Assay – PGM™
9-121-0037 LymphoTrack® Dx *IGH* FR2 Assay – PGM™
9-121-0047 LymphoTrack® Dx *IGH* FR3 Assay – PGM™

9-122-0009 LymphoTrack® Dx *IGK* Assay Kit A – MiSeq®
9-122-0019 LymphoTrack® Dx *IGK* Assay Panel – MiSeq®
9-122-0007 LymphoTrack® Dx *IGK* Assay – PGM™
9-225-0009 LymphoTrack® Dx *TRB* Assay Kit A - MiSeq®
9-225-0019 LymphoTrack® Dx *TRB* Assay Panel - MiSeq®
9-227-0019 LymphoTrack® Dx *TRG* Assay Kit A – MiSeq®
9-227-0009 LymphoTrack® Dx *TRG* Assay Panel – MiSeq®
9-227-0007 LymphoTrack® Dx *TRG* Assay – PGM™
9-500-0009 LymphoTrack® Dx Software – MiSeq®
9-500-0007 LymphoTrack® Dx Software – PGM™

Next-Generation Sequencing Research Use Only LymphoTrack Assays

7-121-0059 LymphoTrack® *IGHV* Somatic Hypermutation Assay Kit A – MiSeq®
7-121-0069 LymphoTrack® *IGHV* Somatic Hypermutation Assay Panel – MiSeq®
7-121-0129 LymphoTrack® *IGH* FR1/2/3 Assay Kit A – MiSeq®
7-121-0139 LymphoTrack® *IGH* FR1/2/3 Assay Panel – MiSeq®
7-121-0009 LymphoTrack® *IGH* FR1 Assay Kit A – MiSeq®
7-121-0039 LymphoTrack® *IGH* FR1 Assay Panel – MiSeq®
7-121-0089 LymphoTrack® *IGH* FR2 Assay Kit A – MiSeq®
7-121-0099 LymphoTrack® *IGH* FR2 Assay Panel – MiSeq®
7-121-0109 LymphoTrack® *IGH* FR3 Assay Kit A – MiSeq®
7-121-0119 LymphoTrack® *IGH* FR3 Assay Panel – MiSeq®
7-121-0057 LymphoTrack® *IGH* FR1/2/3 Assay – PGM™
7-121-0007 LymphoTrack® *IGH* FR1 Assay – PGM™
7-121-0037 LymphoTrack® *IGH* FR2 Assay – PGM™
7-121-0047 LymphoTrack® *IGH* FR3 Assay – PGM™
7-122-0009 LymphoTrack® *IGK* Assay Kit A – MiSeq®
7-122-0019 LymphoTrack® *IGK* Assay Panel – MiSeq®
7-122-0007 LymphoTrack® *IGK* Assay – PGM™
7-225-0009 LymphoTrack® *TRB* Assay Kit A - MiSeq®
7-225-0019 LymphoTrack® *TRB* Assay Panel - MiSeq®
7-227-0019 LymphoTrack® *TRG* Assay Kit A – MiSeq®
7-227-0009 LymphoTrack® *TRG* Assay Panel – MiSeq®
7-227-0007 LymphoTrack® *TRG* Assay – PGM™
7-500-0007 LymphoTrack® Software – PGM™
7-500-0008 LymphoTrack® MRD Software
7-500-0009 LymphoTrack® Software – MiSeq®

Capillary & Gel Fragment Analysis CE-IVD IdentiClone™ Assays

9-100-0010 IdentiClone™ *IGH + IGK* B-Cell Clonality Assay – Gel Detection
9-100-0020 IdentiClone™ *IGH + IGK* B-Cell Clonality Assay MegaKit – Gel Detection
9-100-0031 IdentiClone™ *IGH + IGK* B-Cell Clonality Assay – ABI Fluorescence Detection
9-100-0041 IdentiClone™ *IGH + IGK* B-Cell Clonality Assay MegaKit – ABI Fluorescence Detection
9-101-0020 IdentiClone™ *IGH* Gene Clonality Assay – Gel Detection
9-101-0040 IdentiClone™ *IGH* Gene Clonality Assay MegaKit – Gel Detection
9-101-0061 IdentiClone™ *IGH* Gene Clonality Assay – ABI Fluorescence Detection
9-101-0081 IdentiClone™ *IGH* Gene Clonality Assay MegaKit – ABI Fluorescence Detection
9-102-0020 IdentiClone™ *IGK* Gene Clonality Assay – Gel Detection
9-102-0021 IdentiClone™ *IGK* Gene Clonality Assay – ABI Fluorescence Detection
9-102-0030 IdentiClone™ *IGK* Gene Clonality Assay MegaKit – Gel Detection
9-102-0031 IdentiClone™ *IGK* Gene Clonality Assay MegaKit – ABI Fluorescence Detection
9-103-0010 IdentiClone™ *IGL* Gene Clonality Assay – Gel Detection
9-103-0011 IdentiClone™ *IGL* Gene Clonality Assay – ABI Fluorescence Detection
9-103-0020 IdentiClone™ *IGL* Gene Clonality Assay MegaKit – Gel Detection
9-103-0021 IdentiClone™ *IGL* Gene Clonality Assay MegaKit – ABI Fluorescence Detection
9-200-0010 IdentiClone™ *TCRB + TCRG* T-Cell Clonality Assay – Gel Detection
9-200-0011 IdentiClone™ *TCRB + TCRG* T-Cell Clonality Assay – ABI Fluorescence Detection
9-200-0020 IdentiClone™ *TCRB + TCRG* T-Cell Clonality Assay MegaKit – Gel Detection
9-200-0021 IdentiClone™ *TCRB + TCRG* T-Cell Clonality Assay MegaKit – ABI Fluorescence Detection
9-205-0010 IdentiClone™ *TCRB* Gene Clonality Assay – Gel Detection
9-205-0011 IdentiClone™ *TCRB* Gene Clonality Assay – ABI Fluorescence Detection
9-205-0020 IdentiClone™ *TCRB* Gene Clonality Assay MegaKit – Gel Detection
9-205-0021 IdentiClone™ *TCRB* Gene Clonality Assay MegaKit – ABI Fluorescence Detection
9-206-0010 IdentiClone™ *TCRD* Gene Clonality Assay – Gel Detection
9-206-0011 IdentiClone™ *TCRD* Gene Clonality Assay – ABI Fluorescence Detection
9-206-0020 IdentiClone™ *TCRD* Gene Clonality Assay MegaKit – Gel Detection
9-206-0021 IdentiClone™ *TCRD* Gene Clonality Assay MegaKit – ABI Fluorescence Detection
9-207-0020 IdentiClone™ *TCRG* Gene Clonality Assay – Gel Detection
9-207-0021 IdentiClone™ *TCRG* Gene Clonality Assay – ABI Fluorescence Detection
9-207-0040 IdentiClone™ *TCRG* Gene Clonality Assay MegaKit – Gel Detection
9-207-0041 IdentiClone™ *TCRG* Gene Clonality Assay MegaKit – ABI Fluorescence Detection
9-207-0101 IdentiClone™ T-Cell Receptor Gamma Gene Rearrangement Assay 2.0 – ABI Fluorescence Detection
9-207-0111 IdentiClone™ T-Cell Receptor Gamma Gene Rearrangement Assay 2.0 MegaKit – ABI Fluorescence Detection
9-308-0010 IdentiClone™ *BCL1/J*H Translocation Assay – Gel Detection
9-308-0020 IdentiClone™ *BCL1/J*H Translocation Assay MegaKit – Gel Detection
9-309-0020 IdentiClone™ *BCL2/J*H Translocation Assay – Gel Detection
9-309-0040 IdentiClone™ *BCL2/J*H Translocation Assay MegaKit – Gel Detection

Capillary & Gel Fragment Analysis CE-IVD LeukoStrat® Assays

9-412-0010 LeukoStrat® *FLT3* Mutation Assay - Gel Detection
9-412-0020 LeukoStrat® *FLT3* Mutation Assay MegaKit - Gel Detection
9-412-0091 LeukoStrat® *FLT3* Mutation Assay 2.0 – ABI Fluorescence Detection
9-412-0101 LeukoStrat® *FLT3* Mutation Assay 2.0 MegaKit – ABI Fluorescence Detection
K-412-0291 LeukoStrat® CDx *FLT3* Mutation Assay 33 reactions – ABI Fluorescence Detection
K-412-0281 LeukoStrat® CDx *FLT3* Mutation Assay Software

Analyte Specific Reagents

A-101-0010 *IGH* FR1 – Unlabeled
A-101-0011 *IGH* FR1 – 6FAM
A-101-0020 *IGH* FR2 – Unlabeled
A-101-0030 *IGH* FR3 – Unlabeled
A-101-0031 *IGH* FR3 – HEX
A-101-0041 *IGH* DH1 - 6 – HEX
A-101-0051 *IGH* DH7 – 6FAM
A-101-0061 *IGH* Framework 1 – 6FAM
A-101-0070 *IGH* Framework 2 – Unlabeled
A-101-0080 *IGH* Framework 3 – Unlabeled
A-101-0081 *IGH* Framework 3 – HEX
A-101-0091 *IGH* Framework 2 – 6FAM
A-101-0101 *IGH* FR2 – 6FAM
A-102-0010 *IGK* V - J – Unlabeled
A-102-0011 *IGK* V - J – 6FAM
A-102-0020 *IGK* V - Kde – Unlabeled
A-102-0021 *IGK* V - Kde – 6FAM
A-103-0011 *IGL* V - J – 6FAM
A-205-0010 *TCRB* V - J1+2 – Unlabeled
A-205-0011 *TCRB* V - J1+2 – 6FAM & HEX
A-205-0020 *TCRB* V - J2 – Unlabeled
A-205-0021 *TCRB* V - J2 – 6FAM
A-205-0030 *TCRB* D - J1 +2 – Unlabeled
A-205-0031 *TCRB* D - J1+ 2 – 6FAM & HEX
A-206-0011 *TCRD* V - D - J – 6FAM & HEX
A-207-0021 *TCRG* V(1-8)J – HEX
A-207-0030 *TCRG* V(1-8,10)J – Unlabeled
A-207-0031 *TCRG* V(1-8,10)J – 6FAM & HEX
A-207-0040 *TCRG* V(9,11)J – Unlabeled
A-207-0041 *TCRG* V(9,11)J – 6FAM & HEX
A-207-0071 *TCRG* V(1-8,9)J – 6FAM
A-207-0091 *TCRG* V(2-5,8-11)J1+2+P – 6FAM
A-309-0050 *BCL2/J*H Mbr – Unlabeled
A-309-0060 *BCL2/J*H 3'Mbr – Unlabeled
A-309-0070 *BCL2/J*H mcr – Unlabeled
A-310-0020 *BCR/ABL* e1.1-a3.1 – Unlabeled
A-310-0030 *BCR/ABL* b2.1-a3.1 – Unlabeled
A-310-0051 *BCR/ABL*, e1.2-a2.2 – HEX
A-310-0061 *BCR/ABL*, e1.2-a3.2 – HEX
A-310-0071 *BCR/ABL*, b2.2-a2.2 – 6FAM
A-310-0081 *BCR/ABL*, b2.2-a3.2 – 6FAM
A-310-0101 *BCR/ABL*, b3.2-a3.2 – 6FAM
A-311-0020 *PML/RARa*, MYL2-RARa – Unlabeled
A-311-0030 *PML/RARa*, MYL1-RARa – Unlabeled
A-311-0040 *PML/RARa*, PML3-RARa – Unlabeled

Alphabetical Index

A

B

C

D

E,F,G,H,I

J,K,L

M,N,O,P

Q,R,S

T

U,V,W,X,Y,Z

Notes

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Patent Notice

Many of the products described herein are covered by one or more of the following: European Patent Number 1549764, European Patent Number 2418287, European Patent Number 2460889, Japanese Patent Number 4708029, United States Patent No. 7,785,783, United States Patent 8859748, and related pending and future applications. All of these patents and applications are licensed exclusively to Invivoscribe®. Additional patents licensed to Invivoscribe covering some of these products apply elsewhere, or as described in this catalog.

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