

FlexISH[®]

Products for flexible FISH

Signal Interpretation Guide

FlexISH[®] BCL2/BCL6 DistinguishSH[™] Probe



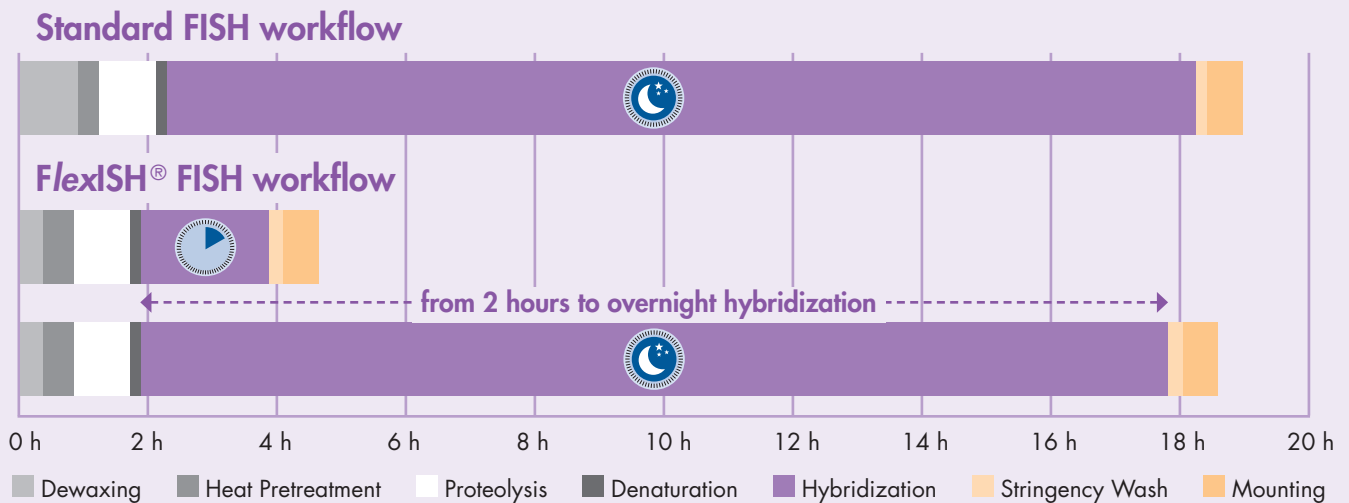
1 Probe · 2 Targets · 3 Answers

One Probe • Two Targets • Flexible Application

The **FlexISH® BCL2/BCL6 DistinguISH™ Probe** is designed to simultaneously detect BCL2 and BCL6 rearrangements in a single nucleus. This innovative probe design enables the user to discriminate between rearrangements affecting the BCL2 and/or the BCL6 gene locus, frequently found in various types of non-Hodgkin lymphomas (NHL). BCL2 and BCL6 rearrangements are known to occur concurrently with MYC rearrangements. Lymphomas with MYC rearrangements and either BCL2 or BCL6 co-aberrations are so-called double-hit B-cell lymphomas (DHL) known to be highly aggressive with poor prognosis. Rarely, triple-hit B-cell lymphomas (THL) occur. According to the revised 4th edition of the WHO classification of tumours of haematopoietic and lymphoid tissues (2017), DHL and THL are classified as high-grade B-cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements. The specific analysis of BCL2 and BCL6 rearrangements in NHL patients is a very effective and reliable tool for the diagnosis and the prediction of the clinical outcome of these patients.^{1, 3}

FlexISH® brings Flexibility to Your FISH

With the use of the **FlexISH® BCL2/BCL6 DistinguISH Probe** in combination with the **FlexISH®-Tissue Implementation Kit** reliable results can be obtained already within **4.5 hours**. The **FlexISH® protocol** can also be incorporated into the routine workflow with overnight hybridization providing the highest flexibility.²



- **FlexISH®** maximizes your flexibility in terms of time and laboratory management. Hybridization time can be varied between **2 hours** and **overnight**.
- With a hybridization temperature of 37°C the **FlexISH® protocol** is fully compatible with routine workflows in pathology laboratories.

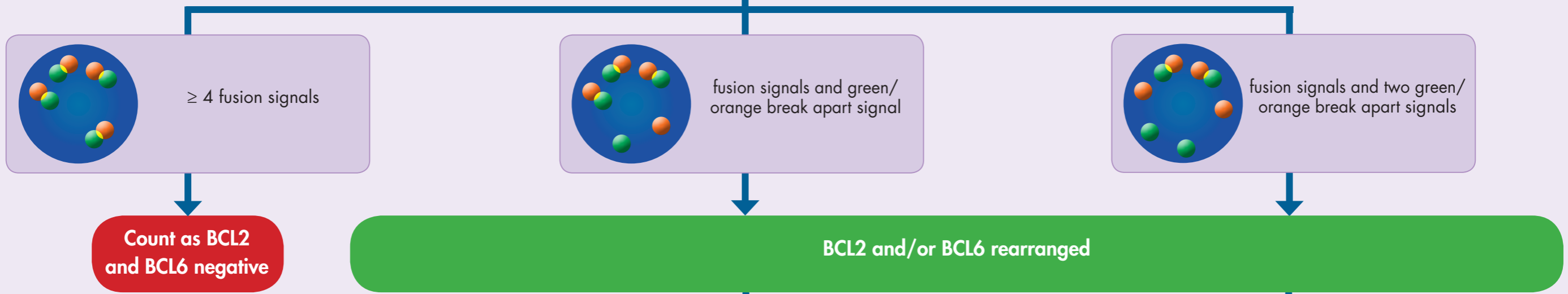
FISH Protocols in Comparison

	ZytoLight®	Dako IQFISH	FlexISH®
Pretreatment	127 min	108 min	103 min
Denaturation	10 min at 75°C	10 min at 66°C	10 min at 75°C
Hybridization	overnight at 37°C	1-2 h at 45°C	flexible between 2 h and overnight at 37°C
Stringency Wash	5 min at 37°C 5 min at 37°C	10 min at 63°C 2x 3 min at RT	10 min at 72°C 3 min at RT
Dehydration & Mounting	33 min	36 min	33 min
Total Time	~ 19 h	~ 4 - 5 h	~ 4.5 h - 19 h

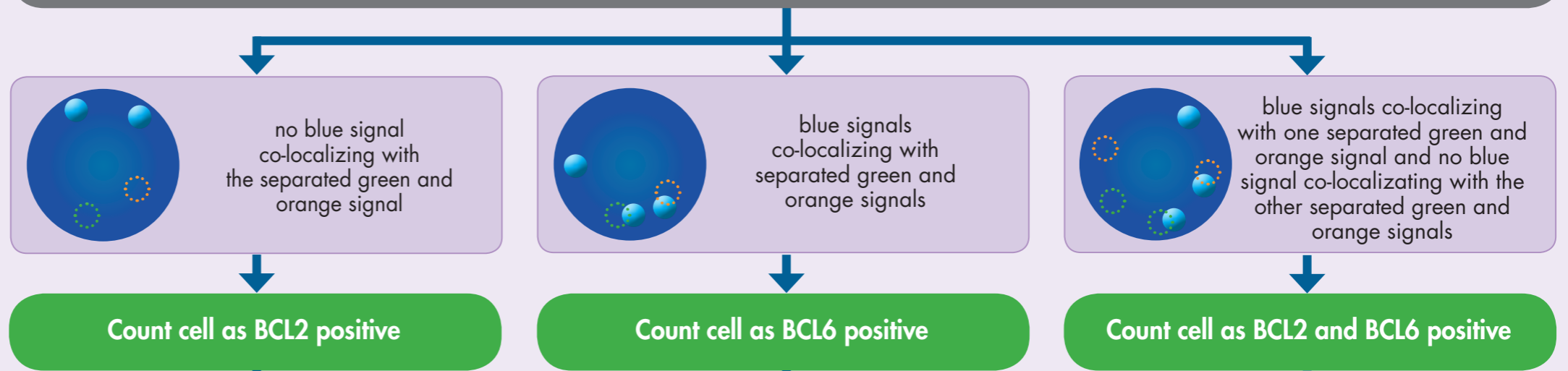
The indicated times include 15 min protease treatment, 15-30 min for the air drying steps, and 16 h for overnight incubation. All other times and temperatures are according to the respective instruction for use.

BCL2/BCL6 Signal Interpretation Guide

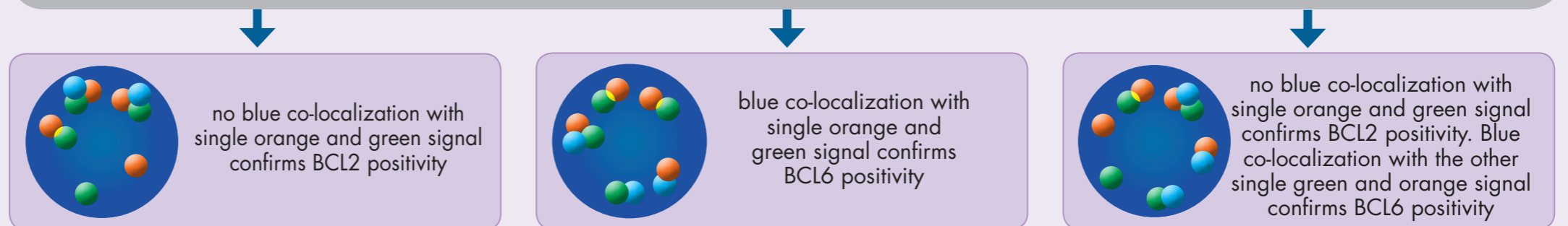
Initial Screening using a Green/Orange Dual Bandpass Filter Set



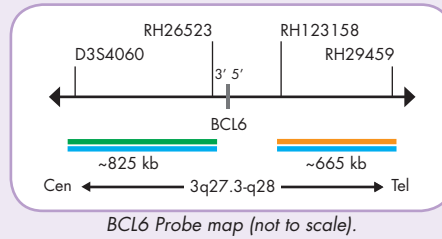
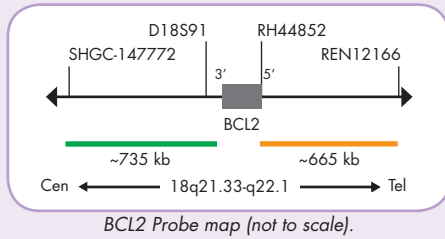
Check for blue Co-Localization using a Blue Single Bandpass Filter Set



Merged Pictures for Clarification



New Multiplex Probe Design to Simplify your FISH



- With the use of the **FlexISH® BCL2/BCL6 Distinguish™ Probe** two genetic targets can be detected simultaneously by performing just **ONE Assay**.
- Less patient material is necessary in order to get reliable results.

Evaluation Procedure

1. The area for counting should include clearly distinguishable and well distributed nuclei.
2. Count at least **50 consecutive and non-overlapping** intact nuclei in an area of a population of tumor cells in the invasive component of the tumor.
3. Determine the BCL2 and BCL6 status according to the **BCL2/BCL6 Signal Interpretation Guide**.
Rearranged if:
 - Distance between the separate green and the separate orange signal is ≥ 2 times the estimated signal diameter
 - **> 15%** of neoplastic cells rearranged*
4. Report if BCL2 or BCL6 status is indeterminate due to, e.g., artifacts, analytic testing failure, etc. or if BCL2 or BCL6 status is discordant with other histopathologic findings and repeat test with another specimen.

*The validation of FISH probes is required for each type of tissue that is intended to be tested in clinical practice since different tissue types exhibit different cell types with different nuclei diameters which may result in different cut off values. In order to correctly interpret the results, the user must validate this product prior to use in diagnostic procedures according to national and/or international guidelines.

The BCL2/BCL6 Distinguish™ Probe · One Probe · Three Answers

The only way to distinguish 3 different gene conditions!

1		Non-rearranged		2 BCL2 specific green/orange (or yellow) fusion signals, and 2 BCL6 specific green/orange/blue fusion signals.
2		BCL2 positivity		Separate orange and green signal, no co-localization with blue signals.
3		BCL6 positivity		Separate orange and green signal co-localizing with blue signals.

FlexISH® BCL2/BCL6 DistinguISH™ Probe

Prod. No.	Product	Label	Tests* (Volume)
Z-2283-50	FlexISH BCL2/BCL6 DistinguISH Probe CE [IVD]	●/●/●	5 (50 µl)
Z-2283-200	FlexISH BCL2/BCL6 DistinguISH Probe CE [IVD]	●/●/●	20 (200 µl)
Related Products			
Z-2182-5	FlexISH-Tissue Implementation Kit CE [IVD] Incl. Heat Pretreatment Solution Citric, 150 ml; Pepsin Solution, 1 ml; 5x FlexISH Wash Buffer, 150 ml; DAPI/DuraTect-Solution, 0.2 ml		5
Z-2182-20	FlexISH-Tissue Implementation Kit CE [IVD] Incl. Heat Pretreatment Solution Citric, 500 ml; Pepsin Solution, 4 ml; 5x FlexISH Wash Buffer, 500 ml; DAPI/DuraTect-Solution, 0.8 ml		20
Z-2099-20	ZytoLight FISH-Cytology Implementation Kit CE [IVD] Incl. Cytology Pepsin Solution, 4 ml; 20x Wash Buffer TBS, 50 ml; 10x MgCl ₂ , 50 ml; 10x PBS, 50 ml; Cytology Stringency Wash Buffer SSC, 500 ml; Cytology Wash Buffer SSC, 500 ml; DAPI/DuraTect-Solution, 0.8 ml		20

* Using 10 µl probe solution per test. CE [IVD] only available in certain countries. All other countries research use only! Please contact your local dealer for more information.

FlexISH®-Tissue Implementation Kit

FlexISH®-Tissue Implementation Kit contains all necessary reagents to perform successful and flexible FISH experiments.

- Heat Pretreatment Solution Citric
- Pepsin Solution
- 5x FlexISH® Wash Buffer
- DAPI/DuraTect™-Solution



FlexISH® Fluorochromes

Two factors that mainly influence FISH analyses:

- Fluorochromes of the FISH probes
- Appropriate filter sets

Fluorochrome	Excitation	Emission	Equivalent to
● ZyBlue™	418 nm	467 nm	DAEC
● ZyGreen™	503 nm	528 nm	FITC
● ZyOrange™	547 nm	572 nm	Rhodamine

Recommended Filter Sets

All filter sets have a superior signal-to-noise ratio and need to be assembled in fluorescence filter holders specific for the respective microscope. Please contact info@zytovision.com for more information.

Prod. No.	Product	Detected Fluorochrome
E-4030-1	DAPI Single Bandpass Filter Set v2	DAPI
E-4026-1	ZyBlue™ Single Bandpass Filter Set v2	●
E-4012-1	ZyGreen™ Single Bandpass Filter Set v2	●
E-4013-1	ZyOrange™ Single Bandpass Filter Set v2	●
E-4016-1	ZyGreen™/ZyOrange™ Dual Bandpass Filter Set v2	●/●

For more product information please contact info@zytovision.com or your local dealer.



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Molecular diagnostics simplified