VIASURE

REAL TIME PCR DETECTION KITS

Our Company

CerTest Biotec is a company focused on the development and manufacture of *in vitro* diagnostic products for human applications.

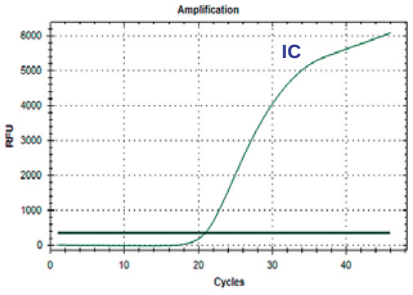
The company was founded in 2002 and its success is highly associated to three basic pillars, our human resources, innovation and customer orientation.

These conditions allow us to provide our customers with products for clinical diagnosis with high standards of quality, using flexible solutions to ensure customer satisfaction.

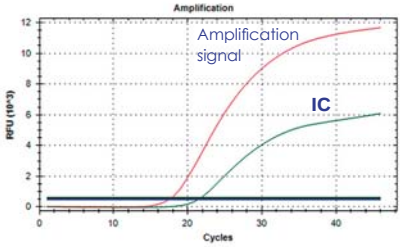
We are backed by our extensive network of distributors in more than 120 countries across the 5 continents.

VS - ATV 113 H

product code | strips or plate | low (L) / high (H) profile



Negative example: no amplification signal



Positive example: amplification signal and Internal Control

Molecular Diagnostics

Molecular detection techniques have revolutionized clinical diagnosis over the past several years. In particular, the Real Time Polymerase Chain Reaction has been turned into one of the most powerful *in vitro* diagnostic tools for the detection and quantification of DNA or RNA.

In this way, the Real Time PCR allows us to identify the causal pathogens of the infectious diseases by the use of specific primers and a fluorescent–labelled probe that hybridizes to a specific region of the target gene.

VIASURE

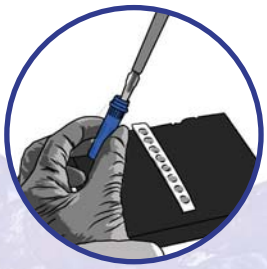
VIASURE® was created with the purpose to provide Molecular Diagnostic users new Real Time PCR kits for the detection of pathogens of interest. VIASURE® provides maximum flexibility and compatibility with leading open system thermal cyclers and the latest technology for the stabilization of products.

VIASURE® Real Time PCR Detection Kits are designed for the diagnosis of infectious diseases caused by different pathogens in human samples. This new product range is based on 5´ nuclease chemistry. This technology utilizes two primers and a hydrolysis probe and exploits the exonuclease activity of Taq DNA polymerase. During the amplification, the increase in the fluorescent signal is proportional to the quantity of target sequence present in the sample and could be measured on a wide range of Real Time PCR platforms.

VIASURE® Real Time PCR Detection Kit contains in each well all the necessary components for Real Time PCR assay in a stabilized format, which allows the shipment and storage at room temperature.

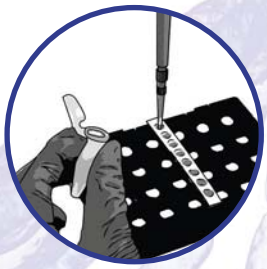
Work Flow

Determine and separate the number of required reactions including samples and controls. One positive and negative control must be included in each run. Peel off protective aluminium seal from plates or strips.



Step 1

Add 15 µl of rehydration buffer into each well.



Step 2

Add 5 µL of:

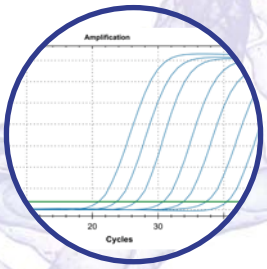
· DNA/RNA sample,

· or Positive control,

· or Negative control.

Step 3

Load the strips into the thermocycler and run the specified protocol.



Step 4

Interpretate results.

Components

Reagent / Material	Description	Quantity
8-well strips	A mix of enzymes, primers-probes, buffer, dNTPs, stabilizers and internal control in stabilized format	6/12 x 8-well strip
96-well plate	A mix of enzymes, primers-probes, buffer, dNTPs, stabilizers and internal control in stabilized format	1 plate
Rehydration Buffer	Solution to reconstitute the stabilized product	1 vial x 1,8 mL
Positive Control	Non-infectious synthetic lyophilized DNA/cDNA	1 vial
Negative Control	Non template control	1 vial x 1 mL
Water RNAse/DNAse free	Water RNAse/DNAse free	1 vial x 1 mL
Tear-off 8-cap strips	Optical caps for sealing wells during thermal cycling	6/12 x 8 cap strip
Shell Frame Grid	Shell Frame Grid	1 or 2

Components included for each VIASURE Real Time PCR Detection Kit.

Kit References

Reference	Description
VS - *XXX*106L	Viasure (*name of the product*) Real Time PCR Detection Kit 6 x 8-well strips, low profile
VS - *XXX*106H	Viasure (*name of the product*) Real Time PCR Detection Kit 6 x 8-well strips, high profile
VS - *XXX*112L	Viasure (*name of the product*) Real Time PCR Detection Kit 12 x 8-well strips, low profile
VS - *XXX*112H	Viasure (*name of the product*) Real Time PCR Detection Kit 12 x 8-well strips, high profile
VS - *XXX*113L	Viasure (*name of the product*) Real Time PCR Detection Kit 96-well plate, low profile
VS - *XXX*113H	Viasure (*name of the product*) Real Time PCR Detection Kit 96-well plate, high profile

Change *"XXX"* for the reference code of the selected kit.

Benefits and Advantages

- Ready-to-use Kits, containing all the necessary components for the testing.
- **Transport and storage at room temperature.**
- **Shelf-life: 24 months** -for all our qPCR products-.
- Easy-to-use. It minimizes the number of manipulations reducing time and possible errors.
- High sensibility, specificity and reproducibility. It allows the identification of pathogens at the early stages of the infection.
- Versatile. Possibility to analyze from 1 up to 96 samples per assay.
- Flexible. Adaptable to a high number of commercial thermal cyclers.
- Evaluated and validated according to ISO 13485 and CE marked.

Compatibilities

Low Profile Block Thermocyclers	
Manufacturer	**Model**
Qiagen	Rotor-Gene® Q*
Cepheid	SmartCycler® *
DNA-Technology	DTprime Real-time Detection Thermal Cycler
DNA-Technology	DTlite Real-time PCR System
Applied Biosystems	7500 Fast Real-time PCR System
Applied Biosystems	7500 Fast Dx Real-Time PCR System
Applied Biosystems	QuantStudio™ 12K Flex 96-well Fast
Applied Biosystems	QuantStudio™ 6 Flex 96-well Fast
Applied Biosystems	QuantStudio™ 7 Flex 96-well Fast
Applied Biosystems	QuantStudio™ 3 Real-Time PCR System
Applied Biosystems	QuantStudio™ 5 Real-Time PCR System
Applied Biosystems	StepOne Plus™ Real-Time PCR System
Applied Biosystems	StepOne™ Real-Time PCR System
Applied Biosystems	ViiA™ 7 Fast Real-Time PCR System
Bio-Rad	CFX96 Touch™ Real-Time PCR Detection System
Bio-Rad	Mini Opticon™ Real-Time PCR Detection System
Roche	LightCycler® 480 Real-Time PCR System
Roche	LightCycler® 96 Real-Time PCR System
Agilent Technologies	AriaMx Real-Time PCR System
BIONEER	Exicycler™ 96 (Fast block)

High Profile Block Thermocyclers	
Manufacturer	**Model**
Qiagen	Rotor-Gene® Q*
Cepheid	SmartCycler® *
DNA-Technology	DTprime Real-time Detection Thermal Cycler
DNA-Technology	DTlite Real-time PCR System
Applied Biosystems	7300 Real-time PCR System
Applied Biosystems	7500 Real-Time PCR System
Applied Biosystems	7900 HT Real-Time PCR System
Applied Biosystems	ABI PRISM 7000
Applied Biosystems	ABI PRISM 7700
Applied Biosystems	QuantStudio™ 12K Flex 96-well
Applied Biosystems	QuantStudio™ 6 Flex 96-well
Applied Biosystems	QuantStudio™ 7 Flex 96-well
Applied Biosystems	QuantStudio™ 3 Real-Time PCR System
Applied Biosystems	QuantStudio™ 5 Real-Time PCR System
Applied Biosystems	Viia™ 7 Real-Time PCR System
Bio-Rad	CFX96 Touch™ Deep Well Real-Time PCR Detection System
Bio-Rad	iCycler iQ™ Real-Time PCR Detection System
Bio-Rad	iCycler iQ™ 5 Real-Time PCR Detection System
Bio-Rad	MyiQ™ Real-Time PCR Detection System
Bio-Rad	MyiQ™ 2 Real-Time PCR Detection System
Eppendorf	Mastercycler™ ep *realplex*
Stratagene / Agilent Technologies	Mx3000P™ Real-Time PCR System
Stratagene / Agilent Technologies	Mx3005P™ Real-Time PCR System
Analytik Jena Biometra	TOptical
Analytik Jena Biometra	qTOWER 2.0
Abbott	Abbott m2000 RealTime System
BIONEER	Exicycler™ 96 (Normal block)

Compatibilities for monoplex VIASURE Kits. For multiplex kits compatibility, see instructions for use.

* The product should be reconstituted following the appropiate procedure and transferred into specific Rotor-Gene® or SmartCyclers® tubes.

Real Time PCR Initiation Protocol

The objective of this protocol is to facilitate the training of laboratory technicians to perform molecular diagnostic test based on Real Time PCR technology or qPCR from clinical samples. The use of the"RNA-DNA VIASURE Extraction Kit" and "VIASURE Pathogen Real Time PCR Detection Kit" in combination with the signs and symptoms presented by the patient, will allow health personnel to stablish a clinical diagnosis.

For the right development of the mentioned activities, it is necessary to adapt the facilities with adequate equipment and stablish different areas where every stage of the process is going to be done. It is recommended to keep an unidirectional workflow.

Molecular diagnosis unit consist of 4 areas:

Nucleic acids extraction area	Pre-PCR area	"Set-up" PCR area	Amplification and results interpretation area
Sample processing. DNA/RNA extraction with a manual kit or automatic method (optional). 1	Reconstitute the required number of wells. Add negative control. 2	Reconstitute lyophilized positive control. Add extracted DNA/ RNA from each sample. Add positive control. Close the wells with optional caps. 3	Place the strip/plate in qPCR equipment. Program the thermal cycler and start the protocol. Analysis of results with the software installed in the equipment. 4

Recommendations

Real Time PCR or qPCR is a high sensitive and specific tool for molecular diagnostic, so it is extremely necessary to keep standards of hygiene, order and security, in order to avoid possible contaminations that could seriously affect the right development of the test.

Molecular Biology Laboratory staff must have exclusive clothing for each area.

Frequent use of disinfectants at the early stage of the process and after using the equipment.

- Commercial disinfectant solution.
- 70% ethanol.

1 Nucleic acids extraction area

The first area aims to process potentially infectious samples and subsequent extraction and purification of nucleic acids with the "VIASURE RNA-DNA Extraction Kit". Thus, this area is set as biohazard area and is recommended to place all the equipment in close proximity to minimize the displacement of the staff.

You can use your optimized manual or automatic system. Follow the manufacturer's instructions, or ask for more information about RNA-DNA extraction protocol for qPCR assay.

2 Pre-PCR area

Pre-PCR area should be maintained free from any biological material to avoid possible contamination that could interfere with test results.

Basic equipment:

- Laminar flow cabinet.
- PPE equipment: dust free disposable gloves, lab coat, mask, cap.
- Micropipette (2 - 20 µL) (exclusive use for rehydrating the wells and adding the negative control).
- Filter tips.
- Scissors.
- 0.1 - 0.2 ml test tube racks.

"VIASURE Pathogen Real Time PCR Detection Kit" is used in this area.

- Separate the required number of reactions/ wells (including samples and the two saved wells for the positive and negative controls that must always be included in each assay).

- If necessary, cut the required number of wells with scissors.
- Remove the protective aluminium seal.

- Reconstitute each one of the wells with 15 µL of rehydration buffer (blue vial).
- Add 5 µL of negative control (purple vial) in the reserved well for negative control.

After this process, move the rehydrated wells to the set "Set-Up" PCR area.

3 "Set-up" PCR area

Two independent processes are made in the "Set-up" PCR area:

A. Positive control rehydration.
B. Addition of the nucleic acids and positive control to the rehydrated wells.

Biological risk is not potentially infectious for the laboratory staff, but it is recommended to keep the area clean and disinfected to avoid any contamination that could compromise the test result.

Although it is recommended to keep separate areas (nucleic acids extraction, pre-PCR and "Set-ip" PCR), this area could be shared with the pre-PCR area (area 2) cleaning properly the cabinet between both processes and differentiating the use of the pipettes (1st pipette 2 - 20 µL: use for the rehydration buffer and negative control, 2nd pipette 2 - 20 µL: use for nucleic acids and positive control).

Basic equipment:

- Laminar flow cabinet.
- PPE equipment: dust free disposable gloves, lab coat, mask, cap.
- Micropipette (2 - 20 µL) (exclusive use for the addition of nucleic acids and positive control).
- Filter tips.
- Scissors.
- 0.1 - 0.2 ml test tube racks.

"VIASURE Pathogen Real Time PCR Detection Kit" is used in this area.

A. Positive Control Rehydration

The "VIASURE Pathogen Real Time PCR Detection Kit" contains a positive control that must be rehydrated before using in the assay. Since it contains a high number of template copies of the pathogen, it is recommended to open and manipulate before starting with the assay. After that, clean the area with disinfectant solutions and/or 70% ethanol.

- Reconstitute the positive control (red vial) with 100 µL of RNAse/DNAse free water (white vial) and mix with the help of the vortex.
- Divide in aliquots to minimize the freeze-thaw cycles.
- Store at -20ºC after re-suspension.

B. Addition of the nucleic acids and positive control to the rehydrated wells.

- Add 5 µL of extracted DNA/RNA from each sample in the rehydrated wells (with the exception of the two wells reserved for the positive and negative control).

- Add 5 µL of reconstituted positive control (red vial) in the reserved well for positive control.

- Divide the required optical caps number and close the wells.
- If necessary, cut the required optical caps number with a scissor. Be careful not to touch inside with the gloves.

After this process, move the plate/strip to the Real Time PCR equipment.

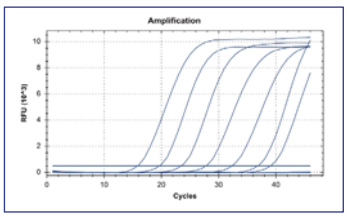
4 Amplification and results interpretation area

This area is where the equipment and related components are located. It is recommended to place this area close to the "Set-up" to minimize movements.

Basic equipment:

- Real Time PCR equipment.
- Computer.

"VIASURE Pathogen Real Time PCR Detection Kit" is used in this area.



- Place the plate/strip in the thermocycler.
- It is recommended to place the same number of plates/strips at both sides of the thermocycler block to balance the equipment.
- Program the thermocycler and start protocol.

DNA THERMAL CYCLING CONDITIONS

Cycles	Time	Temperature
1	2 min	95 °C
45	10 s	95 °C
	50 s	60 °C *

* Data collection

RNA THERMAL CYCLING CONDITIONS

Cycles	Time	Temperature
1	15 min	45 °C
1	2 min	95 °C
45	10 s	95 °C
	50 s	60 °C *

Analysis of results with the software installed in the equipment

Pathogen	Internal Control	Negative Control	Positive Control	Interpretation
+	+/- *	-	+	Positive pathogen
-	+	-	+	Negative pathogen
+	+	+	+	Invalid
-	-	-	-	Invalid

** Detection of internal control is not necessary for highly positive samples. High copy number of target in the sample can lead to a weak or absent signal in the internal control.*

CerTest Biotec, S.L.
Pol. Industrial Río Gállego II · Calle J, Nº1
50840, San Mateo de Gállego, Zaragoza (Spain)
www.certest.es

VIASURE online

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