Dengue virus Real-time PCR Kit

(For Qualitative Detection)

REF 8013

INTENDED USE

Dengue virus Real-time PCR Kit is an in vitro nucleic acid amplification kit for the detection and quantification of Dengue virus specific RNA.

KIT CONTENTS

Components	Number of reaction	Number of vials	Volume Per vials
One step RT-PCR Master Mix	8µl	1	200µl
RT-Taq enzyme mix	2µl	1	50µl
Dengue virus Primer Probe Mix [Dengue PP mix]	2.5µl	1	65µl
Internal control Primer Probe Mix [IC PP Mix]	2.5µl	1	65µl
Internal control template [IC template]	5µl	1	125µl
Internal control template [IC template]	10µl	1	150µl
Internal control template [IC template]		1	4ml

STORAGE

1. The kit is shipped on gel ice. Upon arrival, all components should be stored in -20°C. They are stable until the expiration date stated on the label.
2. Repeated thawing and freezing should be avoided, as this might affect the performance of the assay.
3. If the reagents are to be used only intermittently, they should be frozen in aliquots. Storage at 2 to 8°C should not exceed a period of 5 hours.

PRODUCT DESCRIPTION

Dengue virus Real-time PCR Kit constitutes a ready-to-use system for the detection of Dengue virus specific RNA using polymerase chain reaction (PCR). It contains reagents and enzymes for the specific amplification of the conserved region of the Dengue viral genome, and for the direct detection of the specific amplicon in FAM channel. In addition, it contains an internal control amplification system to identify possible PCR inhibition and RNA purification efficiency. External positive control is supplied, which can be used as both qualitative and quantitative to determination the amount of viral load.

SPECIFICITY

Dengue virus primer and probe have been designed for the specific and exclusive in vitro quantification of Dengue virus subtype 1 to 4. The target sequence (5'UTR) is highly conserved and sequences in this kit have 100% homology with a broad range of clinically relevant reference sequences based on a comprehensive bioinformatics analysis.

ANALYTICAL SENSITIVITY

The analytical sensitivity is defined as the concentration of RNA molecules (copies/µl) that can be detected with a positivity rate of 95%. The analytical sensitivity was determined by analysis of dilution series of quantified Dengue virus specific pDNA from 0.001copies to 10copies/µl in triplicates. Under optimal PCR conditions, the analytical sensitivity is 0.35 copies per micro liter.

RNA PURIFICATION

If you are using a spin column based sample preparation procedure having washing buffers containing ethanol, it is highly recommended to perform an additional centrifugation step for 3min at approximately 17000 x g (~ 13000 rpm), using a new collection tube, prior to the elution of the RNA.

INTERNAL CONTROL TEMPLATE

Add 5µl of the internal control template to each test sample. Do not add directly to test sample. Add after adding lysis buffer to the test sample [sample/lysis buffer mix]. Complete purification according to the manufacturer's protocols.

The primer and probe present at PCR limiting concentrations which allows multiplexing with the target sequence primers. Amplification of the internal control template does not interfere with detection of the pathogen target gene even when present at low copy number. The Internal control is detected through the HEX channel and gives a CT value of 21 +/-10.

PREPARATION OF STANDARD CURVE DILUTION SERIES:

1. Pipette 90µl of PCR grade water into three 1.5ml micro centrifuge tubes and label as QS2 to QS4.
2. Pipette 10µl of Positive control-QS1 into tube QS2.
3. Vortex thoroughly and spin down briefly.
4. Change pipette tip and pipette 10µl from tube QS2 into tube QS3.
5. Vortex thoroughly and spin down briefly.
6. Repeat steps 4 and 5 to complete the dilution series.
7. Use 10µl per reaction.
8. Prepare every time fresh and use.

Standards	Copies per µl
QS-1	2500000
QS-2	250000
QS-3	25000
QS-4	2500

DETECTION PROTOCOL

- Before use, all kit components need to be thawed completely, mixed by gently inverting and centrifuged briefly.
- Make sure that Positive and Negative control is included in every run.
- Make sure that internal control template is added during DNA purification. If not, pipette 2.5µl of the internal control template directly into the purified DNA.
- Include 0.5 reaction volume for pipetting error while calculating the volume for total number of reactions.

Components	Volume per reaction
One step RT-PCR Master Mix	8µl
RT-Taq enzyme mix	2µl
Dengue virus PP Mix	2.5µl
IC PP Mix	2.5µl
Master Mix Volume	15µl
Purified RNA	10µl
Final reaction volume	25µl

Negative Control setup [NTC]

Add 10µl of PCR grade water.

Qualitative Positive Control setup

Add 10µl of any one of the Positive controls [From QS1 to QS4]

Quantitative Positive controls setup

10µl of all Positive controls prepared from QS1 to QS4.

PROGRAMMING THERMAL CYCLER

Sample volume	25µl
Fluorescence Dyes	FAM & HEX
Passive reference	None
Ramping rate	Default

Cycles	Step	Time	Temp
1	Reverse transcriptase	20min	50ºC
	Taq enzyme activation / Hold	15min	95ºC
45 cycles	Denaturation	20sec	95ºC
	Annealing/Data collection*	20sec**	56ºC
	Extension	20sec	72ºC

Data collection/Acquisition	Targets
FAM	Dengue virus
HEX	Internal control

** Some qPCR machines may require minimum 30sec for data collection; in that case, set to 30sec, this will not affect the performance.

READING THE GRAPH

Step-1 – Internal control Validation

Select the test samples alone for the internal control analysis. Select HEX dye and view the graph of internal control amplification. A successful amplification Ct value must be within Ct 21 +/- 10.

This range indicates NO PCR inhibition in the reaction. Any sample value goes beyond Ct 31 indicates that either sample has some issues in the purification or inhibiting PCR reaction.

Internal control will not get amplified in the negative and positive controls. Ignore a late noise HEX amplification graph in the NTC and Positive control wells.

Step-2 – FAM - Negative and Positive control validation

Select the NTC and Positive control [Qualitative] or Standards wells [Quantitative], select FAM channel, and view the graph of amplification.

The NTC must be flat with no Ct value. If required adjust the threshold value just above the NTC. The PC or Standards must be amplified as per their copy numbers.

NTC justifies NO contamination in the reagent as well as fine pipetting and its environment. PC justifies the reagents storage
conditions and reaction parameters are as prescribed.

Step-3 –FAM - Test Sample status

In FAM channel, select test sample well one by one, analyze the graph/amplification.

QUALITATIVE INTERPRETATION OF RESULTS

Test Sample	Negative Control	Positive Control	Internal Control	Interpretation
Positive	Negative	Positive	Positive	Dengue virus specific RNA detected
Negative	Negative	Positive	Positive	No Dengue virus specific RNA Detected. Sample does not contain detectable amounts of Dengue virus specific RNA.
Negative	Negative	Negative	Negative	Experiment fail
Positive	Positive	Positive	Positive	Experiment fail

Calculating copies or IU per ml

Input the machine indicated copy number into the following formula

$$\text{Result (copies/ml)} = \frac{\text{Result (copies/µl) x Elution Volume (µl)}}{\text{Sample Volume (ml)}}$$

Note:

Elution volume: must be typed in micro liter format, example 30µl, 60µl or 100µl. **Sample volume:** must be typed in milliliter format, example 0.2ml or 0.5ml

LIMITATIONS

A false negative result may occur if inadequate numbers of organisms are present in the sample due to improper collection, transport or handling. Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.

The presence of PCR inhibitors may cause under quantification, false negative or invalid results.

Potential mutations within the target regions of the pathogen's genome covered by the primers and/or probes used in the kit may result in under quantification and/or failure to detect.

As with any diagnostic test, Dengue virus Real-time PCR results need to be interpreted in consideration of all clinical and laboratory findings.

QUALITY CONTROL

In accordance with in house Quality Management System, each lot of Dengue virus Real-time PCR kit is tested against predetermined specifications to ensure consistent product quality.

Index of symbols

Consult instructions for use	LOT Batch code	Tests per kit
IVD For *in vitro* diagnostic use only	Do not re-use	Keep dry
2°C 8°C Temperature limit - 25 °C to - 15 °C	Date of manufacture	Biologial Risks
Manufacturer	Use-by date	

Marketed by:

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