

MBPCR101

Generic Dengue Detection Kit (One-Step Real-Time Probe Based PCR)

Description

Dengue viruses are transmitted by the *Aedes aegypti* mosquito and are among the most important arboviruses because of the high morbidity they cause to humans who inhabit urban communities in the tropical and subtropical regions of the world. There are four antigenically related serotypes of dengue viruses (dengue-1, -2, -3 and -4). A primary infection with any of the four serotypes of dengue viruses usually results in subclinical or self-limited febrile disease. The more severe forms of the disease, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), have been reported in up to 5 to 10% of secondary infections, with case fatality rates as high as 10%. With healthcare becoming increasingly available to a large number of people, there is a greater need for faster molecular detection techniques as the number of samples have increased significantly. Molecular diagnostic systems using Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) for detecting dengue viral RNA in human serum/plasma/blood samples have been shown to be faster assays than cell culture and are highly effective for diagnosing dengue fever cases.

NOTE: HiMedia's Generic Dengue Detection Kit (One-Step Real-Time Probe Based PCR) is for *in-vitro* use only.

Intended Use

Recommended for sensitive and specific detection of Dengue viruses in clinical samples and cultures.

Principle

Real-time polymerase chain reaction, also called quantitative Polymerase Chain Reaction (qPCR) or kinetic Polymerase Chain Reaction, is a laboratory technique based on the principle of PCR. This technique is used to amplify a targeted DNA sequence by use of hydrolysis probes that are short oligonucleotides that have a fluorescent reporter dye attached to the 5' end and a quencher dye to the 3' end. HiMedia's Generic Dengue Detection Kit (One-Step Real-Time Probe Based PCR) is designed to detect the **Dengue virus in FAM channel** with **Internal Control in HEX channel** in a single tube reaction. The kit allows sensitive and specific detection of Dengue virus in a single tube reaction.

Positive control

This is a control reaction using a known template (target pathogen). A positive control is usually used to check that the primers have been designed properly and the PCR conditions have been set up correctly.

Internal Control

This is a control sequence which is amplified in the same reaction tube along with the target sequence (target species) but detected with a different primer (i.e. Multiplex PCR). An internal control is often used to detect the failure of amplification in cases where the target sequence is not amplified.



Registered Office

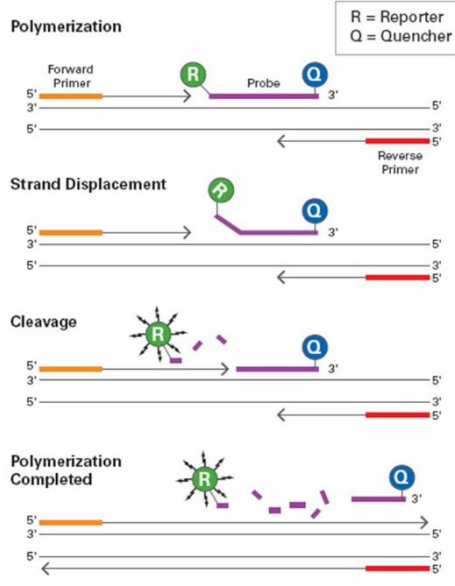
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Diagrammatic representation of preferential binding of probe specific to DNA fragments in Real-time PCR



Polymerization: A fluorescent reporter (R) dye and a quencher (Q) are attached to the 5' and 3' end of the probe respectively

Strand displacement: When the probe is intact, the report dye emission is quenched.

Cleavage: During each extension cycle, the DNA polymerase cleaves the reporter dye from the probe

Polymerization completed: Once separated from the quencher, the reporter dye emits its characteristic fluorescence

While the probe is intact, the proximity of the quencher dye greatly reduces the fluorescence emitted by the reporter dye by fluorescence resonance energy transfer (FRET). The probes are designed such that they anneal within a DNA region amplified by a specific set of primers. During PCR amplification, these probes will hybridize to the target sequences located in the amplicon i.e. the DNA. As the *Taq* DNA polymerase replicates the template with the bound probe, the 5'-nuclease activity of the polymerase enzyme cleaves the fluorescent probe. The end result in cleavage of the probe is separation of the reporter dye from the quencher dye and increasing the reporter dye signal. As the probe is removed from the target strand, primer extension continues to the end of the template strand. Hence, fluorescence detected in the quantitative PCR thermal cycler is directly proportional to the fluorophore released and the amount of DNA template present in the PCR. Thus, inclusion of the probe does not inhibit the overall PCR process.

Features

- Fast and simple
- Good sensitivity and specific results
- Guaranteed reproducible results
- Rapid detection of all relevant clinical pathogens

Types of Specimen: Blood sample / Serum sample / Viral cultures

Specimen collection and Handling

Follow appropriate techniques for handling specimens; after use, contaminated materials must be sterilized by autoclaving before discarding. Standard precautions as per established guidelines should be followed while handling clinical specimens and items contaminated with blood and other body fluids. Safety guidelines may be referred in individual safety data sheets.

Storage and Shelf life

The provided kit has a shelf-life of 12 months when stored at -20°C. Repeated thawing and freezing of PCR reagents should be avoided, as this may reduce the sensitivity. If the reagents are to be used multiple times, we recommend storing reagents as aliquots to avoid repeated freeze and thaw. Degradation of sample RNA specimens can also reduce the sensitivity of the assay. HiMedia Laboratories does not recommend using the kit after the expiry date stated on pack.

Kit Contents: The provided PCR kit contains:

Components	Product code	Reagents provided for (reactions)* (µL)	
		25R	50R
RT Buffer	DS0221	135	270
10X solution H	DS0222	68	135
M-MuLV Reverse Transcriptase	DS0220	27	54
Primer Mix for Generic Dengue	DS0179	54	108
Generic Dengue Probe	DS0199	34	68
Primer Mix for Internal Amplification Control (IAC)	DS0124	27	54
Internal Amplification Control (IAC) Probe	DS0214	34	68
Generic Dengue Positive Control	DS0122	5	10
Nuclease free water	ML065	200	350

* For a 25 µL PCR reaction

Materials needed but not provided:

- PCR tubes (Product code PW1255) or PCR Strips (Product code: PR17) or PCR Plates (Product code: PR2 / PR3 / PR19) & Sealing Film (PR18)
- Insta Q Real Time PCR System (Product Code: LA1012 / LA1023 / LA1024)
- Barrier Micropipette Tips
- Micropipettes

General Preparation Instructions

- Before use all PCR components should be completely thawed on ice (4°C).
- Perform the amplification reactions in a clean area, preferably in a biosafety cabinet.
- Use of aerosol barrier pipette tips is recommended to reduce contamination risks from extraneous RNA templates.
- Extract and store positive control sample (if used) separately from all other reagents to avoid contamination and add it to the reaction mix in a separate area.

A. Protocol for PCR Master Mix Preparation

Components	Volume to be added for 1R (for a 25 µL reaction)
RT Buffer	5 µL
10X solution H	2.5 µL
M-MuLV Reverse Transcriptase	1 µL
Primer Mix for Generic Dengue	2 µL
Generic Dengue Probe	1.25 µL
Primer Mix for Internal Amplification Control (IAC)	1 µL
Internal Amplification Control (IAC) Probe	1.25 µL
Nuclease free water	6 µL
Template RNA/Negative Control	5 µL
Total volume	25 µL

NOTE: (Optional) – The user can also set up an additional PCR reaction containing 1 µL Positive Control + 4 µL of Nuclease free water in a separate tube.

Centrifuge the tube briefly at 6000 rpm for about 10 seconds. Place the tubes in Real-time PCR machine and set the recommended PCR program (mentioned below). Interpret the data from the amplification plot (observe the Ct values).

B. Recommended PCR program

- | | | |
|--------------------------|-------------------------------------|---------------------|
| 1. Reverse Transcription | : 50°C for 15 minutes | |
| 2. Initial denaturation | : 95°C for 2 minutes and 30 seconds | |
| 3. Denaturation | : 95°C for 15 seconds | } No. of cycles: 40 |
| 4. Annealing & Extension | : 60°C for 30 seconds (Plate Read) | |
| Plate Read | : FAM/HEX | |
| 5. Hold | : 4°C for ∞ | |

Warning and Precautions

Read the procedure carefully before beginning the protocol. Wear protective gloves/protective clothing/eye protection/face protection. Follow good clinical laboratory practices while handling clinical samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred in safety data sheets of the product.

Limitations

Although rare, mutations within the highly conserved regions of the targets genes covered by the kit's primers and/or probe may result in under quantitation or failure to detect the presence of the target regions in these cases. Validity and performance of the assay design are revised at regular intervals.

Performance and Evaluation

Each lot of HiMedia's Generic Dengue Detection Kit (One-Step Real-Time Probe Based PCR) is tested against predetermined specifications to ensure consistent product quality.

Quality Control

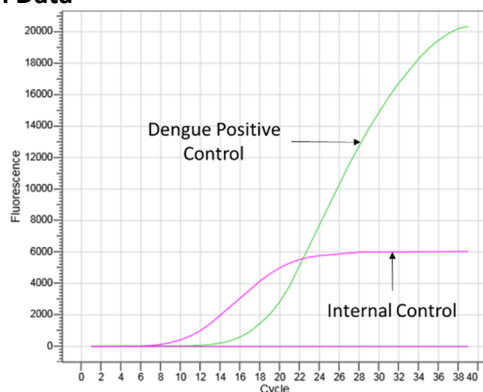
Each lot of HiMedia's Generic Dengue Detection Kit (One-Step Real-Time Probe Based PCR) is assayed for contaminating endonuclease, exonuclease and non-specific RNase activities. Functionally tested in RNA amplification.

Data Analysis

The following conditions should be met for a valid diagnostic test:

Control	Detection channel	
	FAM (Dengue generic)	HEX (Internal Control)
Positive Control	+	+
Negative Control	-	+

Amplification Data



Sr. No.	Sample	C _t value
1.	Dengue Positive Control	15.65
2.	Internal Control	8.68

Image representing probe based real-time amplification data of Dengue virus with C_t values (provided in table)

Data Interpretation

Detection Channel		Result Interpretation
FAM (Dengue generic)	HEX (Internal Control)	
≤ 35 Ct	≤ 35 Ct*	Positive for Dengue generic
-	≤ 35 Ct	Negative for Dengue generic
-	-	PCR inhibition or reagent failure. Repeat PCR or repeat extraction from original sample

*The presence or absence of a signal in the HEX channel is not relevant for the validity of the test run due to competition between the test template and Internal Control template.

Troubleshooting Guide

Sr. No.	Problem	Cause	Solution
1.	No amplification	Degraded samples	1. Check the integrity of RNA using agarose gel electrophoresis. 2. Use freshly prepared RNA to ensure the availability of intact template sequence for efficient amplification.
		Error in protocol setup	Verify that the correct reagent volumes, dilutions and storage conditions have been used.
2.	Variability between replicates	Error in reaction set-up	Prepare a large volume master mix, vortex thoroughly and aliquot into reaction tubes.
		Air bubbles in reaction mix	Briefly centrifuge reaction samples/plate prior to running on a real-time PCR instrument.
		Pipetting error	C _t values of replicates can show increased variation due to poor laboratory technique or imprecise pipettes.
3.	Amplification in negative control	Reagents contaminated	1. Replace all critical solutions. 2. Repeat the analysis of all tests with fresh aliquots of critical reagents.
4.	No signal with positive controls	Incorrect programming of the temperature profile of the thermocycler	Compare the temperature profile to the manual.

Safety Information

HiMedia's Generic Dengue Detection Kit (Real-Time Probe Based PCR) is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques.

Technical Assistance

At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com.



In vitro diagnostic medical device



CE Marking



Storage temperature



Do not use if package is damaged



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