MBPCR137 Hi-PCR® Dengue Serotyping Probe PCR Kit

Instructions For Use

Description

Dengue virus (DENV) is a single-stranded RNA virus comprising mainly four distinct serotypes (DEN-1 to -4). These closely related serotypes of the dengue virus belong to the genus Flavivirus, family Flaviviridae, and are transmitted by *Aedes* spp. mosquitoes. After an incubation period of 4-10 days, infection by any of the four virus serotypes can produce a wide spectrum of clinical manifestations spanning asymptomatic infection, dengue fever (DF), and severe dengue, a category that includes entities previously classified as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Given the geographic expansion of DENV1-4, rapid and accurate serotyping of DENV is crucial for dengue diagnosis and epidemiologic surveillance, treatment of patients, control of DENV outbreaks and transmission blocking strategies targeting the vector, as well as for development of vaccines and antivirals. Nucleic acid amplification-based assays or Polymerase Chain Reaction (PCR) is an alternative method of Dengue serotyping that allows for sensitive and specific detection of Dengue RNA. Real-Time PCR technique is considerably simple and fast with respect to the standard PCR technique. This technique has been successfully used for the rapid detection and identification of a variety of infectious pathogens.

NOTE: HiMedia's Hi-PCR® Dengue Serotyping Probe PCR Kit is for *in-vitro* use only.

Intended Use

Recommended for sensitive and specific detection of DENV 1-4 serotypes in clinical samples.

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 Hi-PCR® Dengue Serotyping Probe PCR Kit is designed to detect the **polyprotein gene** of **Dengue serotypes 1, 2, 3 and 4 in FAM, JOE, Cy5 and TexasRed channels respectively** with **Internal Control in Cy5.5 channel** in a single tube reaction. The kit allows sensitive and specific detection of Dengue serotypes 1-4 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.

Negative Control

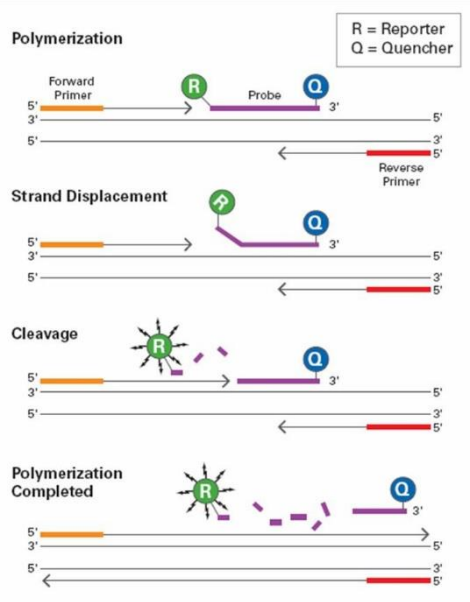
A Negative control is needed to ensure that the reagents, equipment, and environment used in the assay is not contaminated. In this reaction, Nuclease free water is used as the template. It is recommended to have minimum 1 reaction of negative control per run.

Registered Office
HiMedia Laboratories Pvt Ltd.
Plot No. C-40, Road No. 21Y, MIDC, Wagle Industrial Area,
Thane, (West) 400604, Maharashtra, INDIA.
Customer Care No.: 00-91-22-6116 9797
Tel : 00-91-22-6147 1919, 6903 4800
Fax : 6147 1920
Web : www.himedialabs.com
Email : info@himedialabs.com
mb@himedialabs.com

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.

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 polymerae 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

Sample Source: Blood samples / Serum samples / Virus cultures

Storage and Shelf life

The provided kit has a shelf-life of 12 months when stored between -15°C to -25°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 DNA 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	100R
RT Buffer	DS0221	135	270	530
10X solution H	DS0222	68	135	265
M-MuLV Reverse Transcriptase	DS0220	27	54	106
DENV 1-4 Primer-Probe Mix	DS1507	108	216	424
Internal Control Primer-Probe Mix	DS0498	27	54	106
Internal Control DNA	DS1096	27	54	106
Molecular Biology Grade Water for PCR	ML065	200	400	800
DENV 1-4 Positive Control	DS1187	25	50	100

***** For a 25 µL PCR reaction

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.

Sample Preparation

Various samples are routinely examined. For extraction and purification of pure RNA for high yield, perform the nucleic acid purification using HiMedia's extraction kits as instructed in the protocol.

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 / LA1073 / LA1074)
- Barrier Micropipette Tips (Product Code: LA749 / LA749A / LA751 / LA751A / LA750 / LA750A / LA859 / LA859A)
- Micropipettes
- For blood / serum / viral cultures: HiPurA® Viral RNA Purification Kit (MB615)

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 DNA 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	Product code	Volume to be added for 1R (for a 25 µL reaction)
RT Buffer	DS0221	5 µL
10X solution H	DS0222	2.5 µL
M-MuLV Reverse Transcriptase	DS0220	1 µL
DENV 1-4 Primer-Probe Mix	DS1507	4 µL
Internal Control Primer-Probe Mix	DS0498	1 µL
Internal Control DNA	DS1096	1 µL
Molecular Biology Grade Water for PCR	ML065	5.5 µL
Template RNA / Positive Control / Negative Control	-	5 µL
Total volume	-	Upto 25 µL

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 30 seconds	
3.	Denaturation	:	95°C for 15 seconds	No. of cycles: 45
4.	Annealing & Extension	:	61°C for 1 minute (Sampling)	
	Channels	:	FAM/JOE/TexasRed/Cy5/Cy5.5	
5.	Hold	:	4°C for ∞	

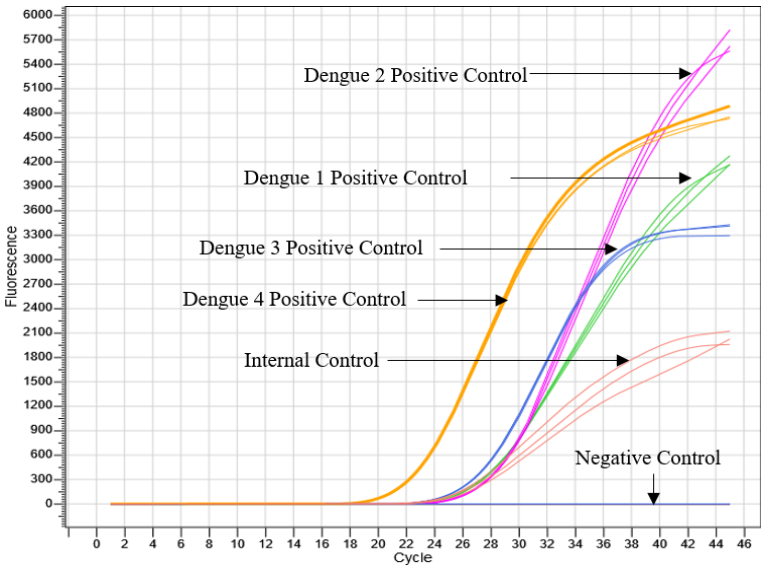
Data Analysis

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

Control	Detection channel				
	FAM (DENV-1)	JOE (DENV-2)	Cy5 (DENV-3)	TexasRed (DENV-4)	Cy5.5 (Internal Control)
Positive Control	+	+	+	+	+
Negative Control	-	-	-	-	+

Target	Ct value	Result
DENV-1	≤ 37	Detected (+)
DENV-2	≤ 38	Detected (+)
DENV-3	≤ 37	Detected (+)
DENV-4	≤ 39	Detected (+)

Amplification Data



Sr. No.	Positive Control	C_t value
1.	DENV-1	29.98
2.	DENV-2	28.44
3.	DENV-3	27.37
4.	DENV-4	22.96
5.	Internal Control	28.68

Image representing amplification plot of DENV 1-4 RNA with Ct values using HiMedia's Hi-PCR® Dengue Serotyping Probe PCR Kit. The results completely depend upon sample types.

Data Interpretation

Target					Result Interpretation
DENV-1 (FAM)	DENV-2 (JOE)	DENV-3 (Cy5)	DENV-4 (TexasRed)	Cy5.5 (Internal Control)	
+	-	-	-	+*	Positive for DENV-1
-	+	-	-	+*	Positive for DENV-2
-	-	+	-	+*	Positive for DENV-3
-	-	-	+	+*	Positive for DENV-4
-	-	-	-	+	Negative for DENV 1-4
-	-	-	-	-	PCR inhibition or reagent failure. Repeat PCR or repeat extraction from original sample

*The presence or absence of a signal in the Cy5.5 channel is not relevant for the validity of the test run due to competition between the test template and Internal Control template.

Limit of Detection (LoD) - Analytical Sensitivity

Sensitivity for the HiMedia's Hi-PCR® Dengue Serotyping Probe PCR Kit was conducted on InstaQ96® Real Time PCR system. The detectable limit of the HiMedia's Hi-PCR® Dengue Serotyping Probe PCR Kit was determined to be 10 copies/µL, 10 copies/µL, 10 copies/µL and 50 copies/µL for DENV-1, DENV-2, DENV-3 and DENV-4, respectively.

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 to in safety data sheets of the product.

Performance and Evaluation

Each lot of HiMedia's Hi-PCR® Dengue Serotyping Probe PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Each lot of HiMedia's Hi-PCR® Dengue Serotyping Probe PCR Kit is assayed for contaminating endonuclease, exonuclease and non-specific DNase activities and has been functionally tested in DNA amplification.

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 Hi-PCR® Dengue Serotyping Probe PCR Kit is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

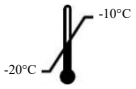
Disposal

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.

Symbols:

	Manufacturer		Do not use if package is damaged
	Authorized representative in the European Community		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		In vitro diagnostic medical device
	Batch code		CE marking of conformity
	Catalogue number		

Authorized representative (AR) Address :

	**AR Experts B.V.** Boeingavenue 209, 1119 PD, Schiphol-Rijk, The Netherlands

Identification No.: PIMBPCR137
Rev.No.: 12
Date of Issue: 2026-01