

MBPCR137A

Hi-PCR® Dengue Serotyping Probe PCR Kit 2.0

Instructions For Use

Description

Dengue virus (DENV) is a single-stranded RNA virus comprising mainly four distinct serotypes (DEN-1, DENV-2, DENV-3 and DENV-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). Many reports have shown an association between DENV genotype and magnitude of the outbreak and disease severity. Since the fatality rate increases severely when a secondary infection occurs by a virus serotype different from that of the initial infection, serotype identification is equally important as virus detection. In recent years, cases of co-circulation of multiple serotypes have also been reported from different parts of world associated with increased disease severity. Given the geographic expansion of dengue, rapid and accurate serotyping of DENV is crucial for 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.

The above reasons underscore the importance of differential typing between DENV viruses. Hi-PCR® Dengue Serotyping Probe PCR Kit 2.0 provides a fast and reliable solution for the Dengue serotyping.

NOTE: HiMedia's Hi-PCR® Dengue Serotyping Probe PCR Kit 2.0 is for *in-vitro* use only.

Intended Use

The Hi-PCR® Dengue Serotyping Probe PCR Kit 2.0 is an in vitro nucleic acid amplification kit for the detection and differentiation of Dengue Virus serotypes DEN-1, DEN-2, DEN-3 and DEN-4 in human biological samples. The kit is intended for use by qualified clinical laboratory personnel trained in the techniques of real-time PCR and in vitro diagnostic procedures.

Principle

The Hi-PCR® Dengue Serotyping Probe PCR Kit 2.0 is based on real-time PCR technology employing reverse transcription of viral RNA template and subsequent dengue specific real-time PCR target amplification 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. Hi-PCR® Dengue Serotyping Probe PCR Kit 2.0 allows simultaneous detection and typing of Dengue serotypes DENV-1, DENV-2, DENV-3 and DENV-4 in a two-tube assay format. Tube 1 includes primers and probes specific for DENV-2, DENV-3 and DENV-4 RNA labelled with the FAM, Texas Red and Cy5 fluorophore respectively. Tube 2 includes primers and probes specific for DENV-1 RNA labelled with the JOE fluorophore respectively along with an endogenous internal control amplification system (probe labelled with fluorophore ROX) to verify efficient viral RNA isolation from clinical samples and PCR amplification.

Controls

Positive control

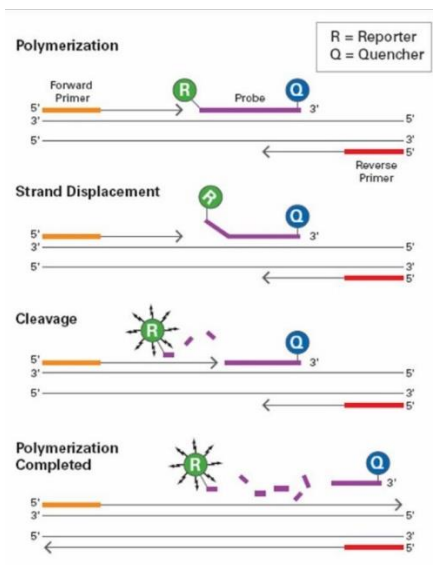
This is a control reaction which contain the target DNA sequence that the PCR is designed to amplify. It is usually used to ensure proper and intended functioning of all the PCR reagents and is recommended to be used in every run to assess optimal performance.

No Template Control

A no template control is needed to ensure that the reagents, equipment, and environment used in the assay are not contaminated with target DNA. In this reaction, PCR grade water is used as the template. It is recommended to have a minimum of one reaction of no template control per run.

Internal Control

This is a control sequence which is amplified in the same reaction tube along with the target sequence from human gene but detected with a different primer. An internal control also allows to check for quality of the clinical specimen, possible PCR inhibition or reagent failure.



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

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

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

Molecular Features:

- Simultaneous serotyping of Dengue viruses (serotypes 1-4) in a single assay.
- Highly sensitive – 1-2 copies/ μ l for the DENV 1-4 serotypes.
- Highly specific – No cross reactivity with other common flavivirus and arbovirus family.

Technology features:

- Fast and reliable results within 90 minutes.
- One-step assay i.e. reverse transcription and amplification are performed in same tube.
- Includes all reagents & controls for validity of the test.
- Open system – Compatible with 4-channel and 5-channel qPCR cyclers.
- Wet-lab assays validated on the Bio-Rad CFX Opus 96, Applied Biosystems QuantStudio 5 and Insta Q96® Plus Real Time PCR Systems.

Types of Specimens and storage

The internal validation of the HiMedia's Hi-PCR® Dengue Serotyping Probe PCR Kit 2.0 was performed using RNA extracted from freshly extracted human serum samples. Other sample materials are not validated. Therefore, we recommend the use of human serum sample for detection of DENV. The RNA should be extracted using a standard viral RNA extraction kit. After collection, serum specimens may be stored at room temperature (15-25°C) for up to 8 hours or refrigerated (2–8°C) for up to 48 hours. Specimens stored beyond these time points must be frozen at or below -20 °C. Frozen specimens should undergo no more than one freeze-thaw cycle. After extraction, store the extracted RNA samples at -20°C for short period storage and -70°C or -80°C for long period storage.

Storage and Shelf life

The provided kit has a shelf-life of 12 months when stored between -15°C and -25°C. Repeated thawing and freezing of PCR reagents should be avoided, not more than 5 freeze-thaw cycles, 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-thaw cycle. Exposure to light, heat or humidity may also affect the shelf life of some of the kit components and should be avoided. Degradation of specimen/ extracted RNA can also hamper the sensitivity of the assay. HiMedia Laboratories does not recommend using the kit after the expiry date stated on pack.

Specimen 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 other body fluids. Safety guidelines may be referred in individual safety data sheets.

Kit Contents:

The provided PCR kit contains:

Components	Product code	Reagents provided for (reactions)* (µL)		
		25R	50R	100R
Dengue Serotyping Mastermix	DS1659	324	636	1272
Dengue Primer-Probe Mix Tube 1	DS1645	81	162	318
Dengue Primer-Probe Mix Tube 2	DS1652	81	162	318
Water	DS0440	486	972	1908
Dengue Positive Control Tube 1	DS1657	81	162	318
Dengue Positive Control Tube 2	DS1658	81	162	318

* For a 30 µL PCR reaction

Materials needed but not provided

Appropriate real-time PCR instrument

Appropriate nucleic acid extraction system or kit

Centrifuge with a rotor for 1.5ml - 2 ml reaction tubes

Centrifuge with a rotor for microtiter plates, if using 96 well reaction plates

Vortex mixer

PCR tubes (0.1ml or 0.2ml) or 96 well reaction plates with corresponding (optical) closing material or lid

Pipettes (Capacity: 0.5 - 10 µL/10 - 100 µL/20 - 200 µL/100 - 1000 µL)

Pipette tips with filters (As per pipette capacity)

Powder-free gloves (disposable)

All these materials are available through www.himedialabs.com

Product name	Product Code
Real-Time PCR Instrument and equipment	
Insta Q96®AG Real time PCR System, 96 well block, 5 channels	MBLA027
Insta Q96®AG 6.0 Real time PCR System, 96 well block, 6 channels	MBLA028

Insta Q96® Plus Real time PCR System, 96 well block, 5 channels	LA1073
Insta Q96® - 6.0 Real time PCR System, 96 well block, 6 channels	LA1074
Insta Q96® Real time PCR System, 96 well block, 5 channels	LA1012
Insta Q48® M4 Real time PCR System, 96 well block, 4 channels	LA1023
Insta Q48® M2 Real time PCR System, 96 well block, 2 channels	LA1024
TabSpin™ Microcentrifuge	LA1089/LA1090
Automated nucleic acid extraction system and materials	
Insta NX® Instrument - fully automated nucleic acid purification system utilizing the Innovative Super -S membrane column method	LA1056
Insta NX® Mag16, Insta NX® Mag16 ^{Plus}	LA1118, MBLA018
Insta NX® Mag32, Insta NX® Mag32 ^{Plus}	LA1096, MBLA019
Insta NX® Mag96	LA1097
Extraction Kits	
HiPurA® Pre-filled Cartridges for Viral Nucleic Acid Purification	MB582PC16
HiPurA® Pre-filled Plates for Viral Nucleic Acid Purification	MB582MPF16
HiPurA® Pre-filled Plates for Viral Nucleic Acid Purification [For Insta NX® Mag32]	MB582MPF-32
HiPurA® Prefilled Plates for Viral Nucleic Acid Purification [For Insta NX® Mag96]	MB582MPF-96
HiPurA® Viral DNA/RNA Purification Kit	MB582
HiPurA® Viral RNA Purification Kit	MB615
Tubes, plates and other consumables	
Varivol II Micropipettes (Capacity: 0.5 to 10 µL/10 to 100 µL/200 to 1000 µL)	LA611/LA614/LA615
µPet Autoclavable Micropipettes (Capacity: 0.5 - 10 µL/10 - 100 µL/20 - 200 µL/100 - 1000 µL)	LA955/LA958/LA959/LA960
Q4Pet Autoclavable Micropipette (Capacity: 0.5 to 10 µL/10 to 100 µL/100 - 1000 µL)	MBLA009/MBLA011/MBLA008
Barrier Tips, Maximum capacity 10 µL	LA749A
Barrier Tips, Maximum capacity 200 µL	LA751A
Barrier Tips, Maximum capacity 1000 µL	LA859A
8-strip tubes & optically clear flat caps for PCR	PR17, PR22, PR23
PCR Tubes, 0.1mL, 0.2 mL; PCR Plates	PW1255/PR2/PR3/PR19
Optical Sealing film	PR18

Kit compatibility with Real-Time PCR Systems

Hi-PCR® Dengue Serotyping Probe PCR Kit 2.0 contains fluorophores that are compatible to the following PCR systems. Hi-PCR® Dengue Serotyping Probe PCR Kit 2.0 however has been validated on Bio-Rad CFX Opus 96, Applied Biosystems QuantStudio 5 and Insta Q96® Plus Real Time PCR Systems.

Real-Time PCR system	Company	Dye 1	Dye 2	Dye 3	Dye 4
Insta Q96® AG/ Insta Q96® AG 6.0/Insta Q96® - 6.0/Insta Q96® Plus/Insta Q48® M4	HiMedia Laboratories Pvt. Ltd.	FAM	HEX	Texas Red	Cy5
BioRad CFX Opus 96/CFX96 Touch/ CFX384 Touch	Bio-Rad Laboratories, Inc.	FAM	JOE/HEX	Texas Red	Cy5
QuantStudio™ 5	Applied Biosystems	FAM	JOE/HEX/VIC	Texas Red/ ROX	Cy5
ABI® Prism SDS 7500	Applied Biosystems	FAM	JOE/HEX/VIC	Texas Red/ ROX	Cy5
QIAquant 96 & 384 5plex	QIAGEN	FAM	JOE/HEX	Texas Red	Cy5
Rotor-Gene®6000 & Q	QIAGEN	FAM	JOE/HEX	Texas Red	Cy5

LightCycler® 96 /LightCycler® 480	Roche	FAM	JOE/HEX/VIC	ROX/ Texas Red	Cy5
qTOWER ³	Analytik Jena	FAM	JOE/HEX/VIC	ROX/ Texas Red	Cy5

Note: Ensure that the Real-Time PCR system is calibrated for dyes mentioned above and maintained according to the manufacturer’s instructions and recommendations.

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.

Protocol for PCR Reaction Mix Preparation

1. In the “Master mix Preparation” area, thaw all components from the kit on ice, mix by inverting the tubes and centrifuge the reagents for 5 seconds. Keep on ice for later use.
2. Based on the number of specimens to be tested (N), calculate the volume of the components to be added as N* volume of “1X”
3. Use 1.5 mL Nuclease free centrifuge tube(s) for the preparation of the PCR reaction mix of Tube 1 and Tube 2. Refer the following table. After all the reagents are added, mix them thoroughly and centrifuge for 5 seconds.

Components	Product code	Volume for “1X” (one reaction)	
		Tube 1	Tube 2
Preparation of PCR Reaction Mix			
Dengue Serotyping Mastermix	DS1659	6 µL	6 µL
Dengue Primer-Probe Mix Tube 1	DS1645	3 µL	----
Dengue Primer-Probe Mix Tube 2	DS1652	----	3 µL
Water	DS0440	6 µL	6 µL
Total PCR Reaction Mix	-	15 µL	15 µL
Template addition			
Template/ Purified Viral RNA	-	15 µL	15 µL
Total reaction volume	-	30 µL	30 µL

4. Aliquot 15 µL of Tube 1 and Tube 2 PCR reaction mix into respective labeled 0.1/0.2mL PCR tube/plate/strips, compatible to the PCR instrument to be used.
5. In the “Nucleic acid handling” area, add 15 µL of extracted RNA of test specimen into the plate/strip to respective wells.
6. For positive and no template control, Template Viral RNA is replaced by Positive control 1 and 2 in tubes 1 and 2 respectively for positive control and by nuclease free water for no template control reaction in both tubes 1 and 2. Refer the following table.

Set up of controls for the PCR run			
Components	Product code	Volume for “1X” (one reaction)	
		Tube 1	Tube 2
Total PCR Reaction Mix	-	15 µL	15 µL
Positive Control			
Dengue Positive Control Tube 1	DS1657	15 µL	-
Dengue Positive Control Tube 2	DS1658	-	15 µL
No Template Control			
Water	DS0440	15 µL	15 µL

Total reaction volume	-	30 µL	30 µL
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7. Tightly cap the tubes/strips or seal the plate using an optically clear adhesive film.
8. Centrifuge the tube briefly at 6000 rpm for about 10 seconds.
9. 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).

Recommended PCR program

Sr. No	Step	Temperature	Time	Sampling	No. of cycles
1.	Reverse Transcription	50°C	15 minutes	---	1
2.	Initial denaturation	95°C	2 minutes 30 seconds	---	1
3.	Denaturation	95°C	15 seconds	---	45
4.	Annealing & Extension	58°C	30 seconds	YES	

Data Analysis

Selection of channels:

	Target	Channels	Quencher
TUBE 1	DENV-2	FAM	None
	DENV-3	Texas Red	None
	DENV-4	Cy5	None
TUBE 2	DENV-1	JOE	None
	Internal Control (IC)	ROX	None

Please select 'Passive reference dye' as 'None' wherever applicable

Data Analysis

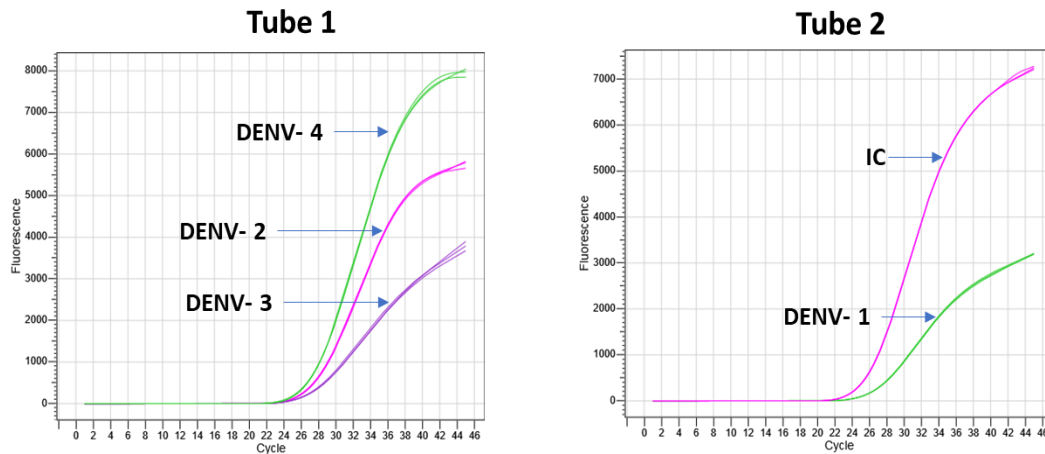
The following conditions should be met for a valid diagnostic test:

Control	Detection channel				
	TUBE 1			TUBE 2	
	FAM (DENV-2)	Texas Red (DENV-3)	Cy5 (DENV-4)	JOE (DENV-1)	ROX (IC)
Positive Control	+	+	+	+	+
No Template Control	-	-	-	-	-

NOTE: For BioRad CFX Opus 96, analysis setting might have to be adjusted. Set the analysis method to "Regression" to obtain Ct values of the test samples (Settings/Cq determination mode/ Regression)

Target	Ct value	Result
DENV-1	≤ 40	Detected (+)
DENV-2	≤ 40	Detected (+)
DENV-3	≤ 40	Detected (+)
DENV-4	≤ 40	Detected (+)

Amplification plot:



Note: Image representing probe based Real-Time amplification of DENV 1-4 serotypes and IC.

Data Interpretation:

Target					Result Interpretation
TUBE 1			TUBE 2		
FAM (DENV-2)	Texas Red (DENV-3)	Cy5 (DENV-4)	JOE (DENV-1)	ROX (IC)	
+	-	-	-	+*	Positive for DENV-2**
-	+	-	-	+*	Positive for DENV-3**
-	-	+	-	+*	Positive for DENV-4**
-	-	-	+	+*	Positive for DENV-1**
-	-	-	-	+	Negative for DENV 1-4
-	-	-	-	-	Inconclusive test*** Likely poor extraction or sample quality. PCR inhibition or reagent failure.

*The presence or absence of a signal in the ROX channel of TUBE 2 is not relevant for the validity of the test run due to competition between the test template and Internal Control template.

** If sample is positive for two DENV serotypes, re-test the specimen. If sample is repetitively positive for both the serotypes, the result may be indicative of a “dual DENV infection” or “co-circulation”.

Please note few cases of co-circulation of three or more DENV serotypes have been reported in the literature however, re-testing the specimen along with confirmation with orthogonal test such as NGS is recommended.

*** When an inconclusive result is obtained, repeat PCR or re-test the extracted RNA or re-extract the specimen and test the newly extracted RNA.

Note: Visually inspect all amplification plots. Do not rely on Ct values alone. Please note that amplification plots exhibit a characteristic sigmoidal shape. Plots that are non-sigmoidal or/and show a sudden rise in fluorescence at very early Ct values (e.g., Ct <6) or plots that are straight line/non-sigmoidal and/or with low fluorescence at late Ct values (e.g., Ct >38) may be due to non-specific amplification, background noise, or instrument artifacts. Such results should be repeated or confirmed before making any clinical interpretation. All negative findings must be correlated with clinical observations and patient history. Negative results do not exclude infections because of other variants of these viruses and should not be used as the sole basis for patient management.

Performance Evaluation

Analytical Sensitivity

Limit of Detection (LoD)

The Limit of Detection (LoD) is defined as the concentration (copies per µl of the eluate) of target molecule that can be detected at 95% or greater probability according to CLSI EP17-A2. The LoD assay of the Hi-PCR®

Dengue Serotyping Probe PCR Kit 2.0 was performed using 20 replicates each on Biorad CFX Opus 96, Applied Biosystems QuantStudio 5 and Insta Q96® Plus Real Time PCR Systems using synthetic nucleic acid for Dengue serotypes DENV-1, DENV-2, DENV-3 and DENV-4. The detectable limit of the Hi-PCR® Dengue Serotyping Probe PCR Kit 2.0 was determined to be 1.9 copies/μL for DENV-1, 1 copy/μL for DENV-2, 1.9 copies/μL for DENV-3 and 1.4 copies/μL for DENV-4.

Analytical Specificity

Inclusivity – In silico

The analytical specificity of the Hi-PCR® Dengue Serotyping Probe PCR Kit 2.0 was ensured by in silico analysis of the oligonucleotides (primers and probes). The oligonucleotide sequences of all the targets were checked by sequence comparison against all the relevant sequences of DENV-1, DENV-2, DENV-3 and DENV-4 available in the GenBank database.

Analytical Reactivity

The analytical reactivity of the Hi-PCR® Dengue Serotyping Probe PCR Kit 2.0 was verified by wet lab testing of the oligonucleotides (primers and probes) against commercially available controls – AMPLIRUN® DENGUE VIRUS RNA CONTROLS from Vircell Microbiologics [DENV 1 (MBC055-R), DENV2 (MBC056-R), DENV3 (MBC057-R), DENV4 (MBC058-R)] and Dengue virus standards from BEI resources- Genomic RNA from Dengue Virus Type 1, Hawaii (NR-4287), Genomic RNA from Dengue Virus Type 2, DENV-2/US/BID-V594/2006 (NR-50531), Genomic RNA from Dengue Virus Type 3, DENV-3/US/BID-V1043/2006 (NR-50532), Genomic RNA from Dengue Virus Type 4, UIS 497 (NR-50533).

Cross-Reactivity and Interference with Other Microorganisms

Wet testing was performed against the genomic or synthetic DNA/RNA of the pathogens (from ATCC) available in the laboratory on Bio-Rad CFX Opus 96 and Insta Q96® Plus Real Time PCR Systems for any potential cross-reactivity. None of the tested pathogen in the below mentioned table showed any reactivity to the primers-probes of the Hi-PCR® Dengue Serotyping Probe PCR Kit 2.0.

Human coronavirus 229E (VR-740D)	Plasmodium falciparum strain 3D7 (PRA-405)
Influenza A virus H1N1 strain A/California/07/2009 (H1N1) pdm09 (VR-1894DQ)	Plasmodium vivax DNA (PRA-3004SD)
Influenza A virus (H3N2) strain A/Wisconsin/15/2009 (VR-1882DQ)	Leptospira interrogans serovar Copenhageni strain Fiocruz L1-130 (BAA-1198D-5)
Influenza B virus strain B/Florida/4/2006 (VR-1804DQ)	Staphylococcus aureus subsp. Aureus (43300DQ)
Hepatitis B virus (VR-3232SD)	Human respiratory syncytial virus strain 18537 (VR-1580DQ)
Hepatitis C virus (VR-3233SD)	Human herpesvirus 1 MacIntyre (VR-539DQ)
Hepatitis A virus (VR-3257SD)	Human herpesvirus 2 (VR-540DQ)
Hepatitis E virus (VR-3258SD)	Human metapneumovirus (VR-3250SD)
Human immunodeficiency virus 1 (HIV-1) RNA (VR-3245SD)	Candida albicans strain SC5314 (MYA-2876DQ)
Middle East respiratory syndrome coronavirus (VR-3248SD)	Mycobacterium tuberculosis strain H37Ra (25177DQ)
Human parainfluenza virus 1 strain C35 (VR-94DQ)	Chlamydomonas pneumoniae strain CM-1 (VR-1360DQ)
Human parainfluenza virus 2 strain Greer (VR-92DQ)	Streptococcus pyogenes strain Bruno (19615DQ)
Human parainfluenzavirus 3 strain C 243 (VR-93DQ)	Mycoplasma pneumoniae strain M129-B7 (29342DQ)
Zika virus (VR-3252SD)	Legionella pneumophila subsp. Pneumophila (33152DQ)
Chikungunya virus (VR-3246SD)	Haemophilus influenzae (51907DQ)
Enterovirus 68 strain Fermon (VR-1826DQ)	Human rhinovirus 16 strain 11757 (VR-283DQ)
Human adenovirus 1 strain Adenoid 71 (VR-1DQ)	

Cross-Reactivity Analysis – in silico

The sequences of the oligonucleotides (primers and probes) in the Hi-PCR® Dengue Serotyping Probe PCR Kit 2.0 were subjected to a BLAST (Basic Local Alignment Search Tool) analysis against the organisms shown in the below table. These organisms were selected if they belonged to Flaviviruses family, were implicated in viral

hemorrhagic fevers, cause Mosquito-borne diseases or could result in acute febrile illness. No significant cross-reactivity was noted for all the subject sequences when evaluated by in-silico Blast analysis.

West Nile virus (taxid:11082)	<i>Brucella melitensis</i> (taxid:29459)
Yellow fever virus (taxid:11089)	<i>Borrelia burgdorferi</i> (taxid:139)
Yellow fever virus group (taxid:40005)	<i>Orientia tsutsugamushi</i> (taxid:784)
Japanese encephalitis virus (taxid:11072)	<i>Salmonella</i> (taxid:590)
Japanese encephalitis virus group (taxid:11071)	<i>Corynebacterium diphtheriae</i> (taxid:1717)
Spondweni virus (taxid:64318)	<i>Burkholderia</i> sp. (taxid:36773)
Spondweni virus group (taxid:297696)	<i>Leptospira</i> (taxid:171)
Tick-borne encephalitis virus (taxid:11084)	<i>Plasmodium</i> (taxid:5820)
Tick-borne encephalitis virus group (taxid:29263)	Hepatitis B virus (taxid:10407)
Zika virus (taxid: 64320)	Hepatitis C virus (taxid:11103)
Chikungunya virus (taxid: 37124)	Hepatitis E virus (taxid:291484)
Modoc virus (taxid:64300)	Human hepatitis A virus (taxid:208726)
Tamana bat virus (taxid:161675)	Human immunodeficiency virus (taxid:11676)
Cell fusing agent virus (taxid:31658)	Cytomegalovirus (taxid:10358)
Omsk hemorrhagic fever virus (taxid:12542)	Epstein-Barr virus (taxid:10376)
St. Louis encephalitis virus (taxid:11080)	Human adenovirus sp. (taxid:1907210)
Murray Valley encephalitis virus (taxid:11079)	Human parvovirus B19 (taxid:10798)
Kyasanur Forest disease virus (taxid:33743)	Monkeypox virus (taxid:10244)
Rift Valley fever virus (taxid:11588)	Wesselsbron virus (taxid:164416)
Israel turkey meningoencephalomyelitis virus (taxid:64291)	Measles morbillivirus (taxid:11234)
Ntaya virus (taxid:64292)	Mumps orthorubulavirus (taxid:2560602)
Tembusu virus (taxid:64293)	Rubella virus (taxid:11041)
Sepik virus (taxid:44026)	Hantavirus (taxid:1980442)
Usutu virus (taxid:64286)	Enterovirus (taxid:12059)
Aroa virus (taxid:64303)	Ebola virus (taxid:1570291)
Bussuquara virus (taxid:64304)	Varicella Zoster Virus (taxid:10335)
Iguape virus (taxid:64308)	agent of lymphatic filariasis (taxid:6279)
Naranjal virus (taxid:64313)	<i>Coxiella burnetii</i> (taxid:777)
Cacipacore virus (taxid:64305)	<i>Staphylococcus aureus</i> (taxid:1280)
Koutango virus (taxid:44025)	<i>Streptococcus pneumoniae</i> (taxid:1313)
Alfuy virus (taxid:44017)	<i>Pseudomonas aeruginosa</i> (taxid:287)
Kunjin virus (taxid:11077)	<i>Streptococcus pyogenes</i> (taxid:1314)
Yaounde virus (taxid:64319)	<i>Burkholderia pseudomallei</i> (taxid:28450)
Kokobera virus (taxid:44024)	<i>Leishmania donovani</i> (taxid:5661)
Stratford virus (taxid:44027)	<i>Listeria monocytogenes</i> (taxid:1639)
Bagaza virus (taxid:64290)	<i>Mycobacterium tuberculosis</i> (taxid:1773)
Ilheus virus (taxid:59563)	<i>Klebsiella pneumoniae</i> (taxid:573)
Rocio virus (taxid:64315)	<i>Rickettsia</i> sp. (taxid:789)
Lymphocytic choriomeningitis virus (taxid:3052303)	<i>Bacillus anthracis</i> (taxid:1392)
<i>Salmonella paratyphi</i> (taxid:54388)	<i>Neisseria meningitidis</i> (taxid:487)
<i>Aspergillus</i> (taxid:5052)	<i>Yersinia pestis</i> (taxid:632)
<i>Histoplasma</i> (taxid:5036)	<i>Acinetobacter baumannii</i> (taxid:470)

Clinical Evaluation

A total of 227 serum samples [117 negative and 110 DENV positive] had been tested to evaluate the clinical performance of the Hi-PCR® Dengue Serotyping Probe PCR Kit 2.0. Overall sensitivity and specificity of Hi-PCR® Dengue Serotyping Probe PCR Kit 2.0 for all targets obtained was 96.4% and 94.9% respectively.

Hi-PCR® Dengue Serotyping Probe PCR Kit 2.0		Real time PCR based Predicate device		
		Positive	Negative	Total
	Positive	106	06	112
Negative	04	111	115	

	Total	110	117	227
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	Estimate (%)	95% CI
Sensitivity	96.4	91.0% to 99.0%
Specificity	94.9	89.2% to 98.1%

Each lot of Hi-PCR® Dengue Serotyping Probe PCR Kit 2.0 is tested against predetermined specifications to ensure consistent product quality.

Extraction kit compatibility:

The following kits and systems are suitable for nucleic acid extraction:

Himedia Viral Purification Nucleic Acid kits MB582, MB615, MB582PC16, MB582MPF16, MB582MPF-32, MB582MPF-96, QIAamp Viral RNA Mini Kit and MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit.

Alternative nucleic acid extraction systems and kits might also be appropriate. The suitability of the nucleic acid extraction procedure for use with Hi-PCR® Dengue Serotyping Probe PCR Kit has to be validated by the user.

Warning and Precautions

Not for Medicinal Use. 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.

Quality Control

Every lot of Hi-PCR® Dengue Serotyping Probe PCR Kit 2.0 would be tested against predetermined specifications to ensure consistent product quality. The Hi-PCR® Dengue Serotyping Probe PCR Kit 2.0 provides controls- Dengue Positive Control (Tube 1 and Tube 2) and a Negative Template Control (NTC) which are to be included in every run.

Limitations

- Strict compliance with the Instructions for Use is required for optimal results and the use of the kit is limited to staff qualified clinical laboratory personnel trained in the techniques of real-time PCR and in vitro diagnostic procedures.
- Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.
- This assay must not be used on the specimen directly. Viral RNA should be extracted from human serum sample using appropriate nucleic acid extraction method.
- Presence of PCR inhibitors and other interferences may lead to false negative or invalid results.
- Although rare, mutations within the highly conserved regions of the targets genes covered by the kit’s primers and/or probe may result in failure to detect the presence of pathogen.
- As with any diagnostic test, results of the Hi-PCR® Dengue Serotyping Probe PCR Kit 2.0 need to be interpreted in consideration of all clinical and laboratory findings.
- Performance of the kit in monitoring treatment of Dengue infection has not been evaluated.

Troubleshooting Guide

Sr. No.	Problem	Possible Cause	Solution
1.	No amplification in test and/or control wells	Degraded samples or poor-quality DNA template	Use freshly extracted, high-quality RNA. Avoid repeated freeze-thaw cycles for stored RNA.
		Missing or incorrect addition of reagents	Verify all reagents were added in the correct volumes and order. Recheck reaction setup steps. Use a master mix to reduce pipetting errors.
		Incorrect thermal cycling conditions	Cross-check the PCR cycling profile with the IFU.
		Incorrect fluorophore/dye setting	Refer the selection of channels table and set the correct fluorophore in the real-time PCR instrument
		Expired or improperly stored reagents	Confirm the expiry date and proper storage. Avoid using reagents that have undergone multiple freeze-thaw cycles
		Instrument malfunction	Verify that the real-time PCR instrument is functioning properly and calibrated.
2.	Variability between replicates	Inconsistent pipetting or error in reaction setup	Prepare a single master mix for all replicates to minimize variation. Vortex thoroughly and aliquot carefully. Use calibrated pipettes and consistent technique.
		Air bubbles in reaction mix	Briefly centrifuge PCR tubes or plate before placing in the instrument to eliminate air bubbles.
		Uneven mixing of reagents	Ensure all reagents are fully thawed and mixed by vortexing before use. Spin down before pipetting.
		Edge effect (thermal variation across plate)	Avoid using outer wells in PCR plates if not temperature-uniform; use a plate seal and consistent plate layout.
3.	Amplification in negative control	Reagent contamination	Replace all critical reagents. Clean workspace, pipettes, and repeat analysis with fresh aliquots. Use filter tips and maintain a unidirectional workflow.
		Cross-contamination or aerosol contamination during reaction setup	Maintain strict unidirectional workflow. Set up reactions in a PCR hood or clean bench. Use aerosol-resistant filter tips for all pipetting steps. Avoid fast, forceful pipetting. Regularly clean work surfaces and equipment with DNA-decontaminating agents. Minimize opening of positive control tubes and avoid splashing.
		Template contamination in workspace or pipettes	Decontaminate work surfaces, pipettes, and equipment using DNA/RNA decontamination solutions. Perform regular cleaning.
		Improper sealing of PCR plate/tubes	Ensure plates/tubes are properly sealed to prevent cross-well contamination during thermal cycling. Use optical-grade seals if required.

4.	No signal with positive control or partial target amplification	Degradation of the positive control material due to improper storage or repeated freeze-thaw cycles	Use a fresh aliquot of positive control. Ensure storage conditions follow IFU and avoid repeated freeze-thaw. Discard expired or compromised controls.
		Incorrect thermal cycling conditions	Cross-verify cycler settings with IFU. Ensure annealing/extension temperature and time are as specified.
		Improper mixing of PC before use	Vortex and briefly spin down the positive control before adding it to the PCR mix. Mix well to ensure homogeneity.
5.	Early Ct value with non-sigmoidal amplification	Instrumental noise, background fluorescence, or non-specific amplification	Visually inspect amplification plots. Disregard flat or atypical (non-sigmoidal) curves that show a sudden rise in fluorescence with very early Ct values (e.g., <6). These may not indicate true amplification. Repeat the assay if necessary. Adjust threshold setting in software within exponential phase. Refer to instrument-specific guidance.
6.	Late Ct value with low RFU and/or non-sigmoidal amplification	Instrumental noise, background fluorescence, or non-specific amplification	Visually inspect amplification plots. Disregard straight line or atypical (non-sigmoidal) curves that show a sudden rise in fluorescence with very late Ct values (e.g., >38) and low RFU. These may not indicate true amplification. Repeat the Extraction with fresh samples and PCR if necessary. Adjust threshold setting in software within exponential phase. Refer to instrument-specific guidance.
7.	Non-specific amplification of targets in samples and/or negative control	Improper threshold setting in the real-time PCR software	Visually inspect all amplification plots. Do not rely on Ct values alone. Manually adjust the threshold as recommended in the threshold setting table. Refer to the instrument-specific guidelines or user manual for proper threshold adjustment.
		Contamination of reagents or workspace	Use fresh aliquots of reagents. Prepare reactions in a contamination-free environment using separate areas for pre- and post-PCR steps. Use aerosol-resistant filter tips and routinely decontaminate surfaces and pipettes.
8.	No Internal Control (IC) amplification	High viral load may suppress IC amplification	If valid Ct for DENV target is observed, it is still considered a valid result. Test repetition with freshly extracted sample is recommended.
		Improper / insufficient sample	If no signals from any of the DENV targets and also from the IC was observed, this indicates improper or insufficient sample collection. Repeat the sample collection.
		Presence of PCR inhibitor	Dilute the extracted nucleic acid in PCR grade water and repeat the PCR assay.

		Sample inhibition, extraction failure, or expired reagents	Repeat extraction or test with fresh sample. Check IC amplification to rule out PCR inhibition. Use validated extraction method and fresh reagents.
		Instrument malfunction	Verify that the real-time PCR instrument is functioning properly and calibrated.
9.	High Ct values in positive samples (late amplification)	Low viral load or suboptimal sample	Results near the cut-off should be interpreted with caution. Repeat test using freshly extracted RNA. Confirm with orthogonal test if clinically significant.
10.	Signal in only one replicate (of duplicate or triplicate reactions)	Pipetting error or borderline positivity	Repeat the test. If consistent upon retesting, interpret cautiously in context of clinical findings. Borderline cases (close to Ct cutoff) may require repeat sampling or orthogonal testing (e.g., NGS). Use calibrated pipettes and proper technique.




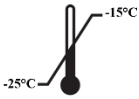





Safety Information

HiMedia’s Hi-PCR® Dengue Serotyping Probe PCR Kit 2.0 is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

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
	Batch code		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		Catalogue number
	In vitro diagnostic medical device		

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Disclaimer :

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