MBPCR179 Hi-PCR® Dengue-Chikungunya Multiplex Probe PCR Kit

Description
Arboviral infections are important public health concerns worldwide. For the countries, where there is local transmission of dengue and chikungunya, it is imperative to effectuate range of technical factors that promote prevention and restrict activity of the causative agents, as they share nearly same clinical manifestations during the first week of infection and poses diagnostic challenge to the treating clinicians. Dengue and chikungunya are important mosquito-borne diseases and endemic in India. Each of these diseases contributes substantially to the morbidity, if not diagnosed and managed at an early stage. Fever, joint pain, headache, vomiting and fatigue are common denominator symptoms of dengue and chikungunya with different clinical outcomes. Concurrent infections with these infective agents cause overlapping of clinical features leading to diagnostic challenge by the physicians. Coinfections with dengue and chikungunya have been reported globally.

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

Intended Use: Recommended for sensitive and specific detection of dengue and chikungunya 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-Chikungunya Multiplex Probe PCR Kit is designed to detect the specific regions of Dengue in FAM channel, Chikungunya in HEX channel with Internal Control in Cy5 channel 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.

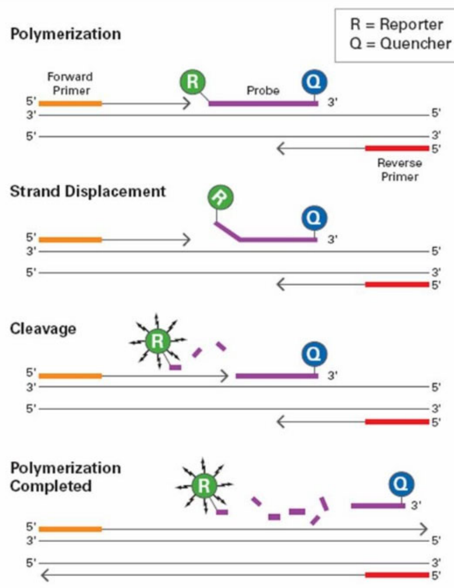
Internal Control
This is a control sequence which is amplified in the same reaction tube along with the target sequence (target species) but detected with a different primer (i.e. Multiplex PCR). An internal control is often used to detect the failure of amplification in cases where the target sequence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

ISO 9001-2015 CERTIFIED WHO GMP CERTIFIED ISO 13485-2016 CERTIFIED

Registered Office
HiMedia Laboratories Pvt Ltd.
Plot No. C-40, Road No. 21Y, MIDC, Wagle Industrial Estate, 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

The information contained herein is believed to be accurate and complete. However no warranty or guarantee whatsoever is made or is to be implied with respect to such information or with respect to any product, method or apparatus referred to herein

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

Sample Source: Blood sample / Serum sample

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 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 * (mL)	
		25R	50R
Hi-Quanti 5X OneStep Mastermix	MBT199	0.108 mL	0.216 mL
Dengue-Chikungunya Primer-Probe Mix	DS1144	0.135 mL	0.270 mL
Dengue-Chikungunya Positive Control	DS1150	0.05 mL	0.10 mL
Water	DS0440	0.2 mL	0.4 mL

* For a 20 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 / LA1023 / LA1024 / LA1073 / LA1074)
- Barrier Micropipette Tips (Product Code: LA749 / LA749A / LA751 / LA751A / LA750 / LA750A / LA859 / LA859A)
- Micropipettes
- Viral RNA extraction: 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 20 µL reaction)
Hi-Quanti 5X OneStep Mastermix	MBT199	4 µL
Dengue-Chikungunya Primer-Probe Mix	DS1144	5 µL
Water	DS0440	1 µL
Template RNA/Negative Control/Positive Control	-	10 µL
Total volume	-	20 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. cDNA Synthesis : 55°C for 15 minutes
2. Initial denaturation : 95°C for 30 seconds
3. Denaturation : 95°C for 10 seconds
4. Annealing : 55°C for 1 minute (Sampling)

   Channels : FAM/HEX/Cy5

(Steps 3–4 and Channels) No. of cycles: 45

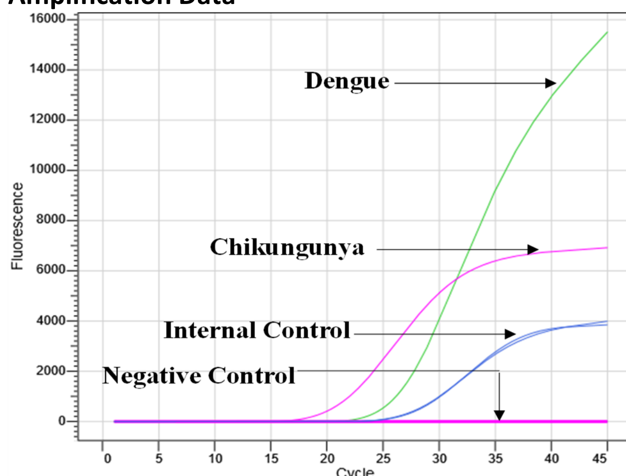
Data Analysis

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

Control	Detection Channel		
	FAM (DENV)	HEX (CHKV)	Cy5 (Internal Control)
Positive Control	+	+	+
Negative Control	-	-	+

Ct Cut-off value	Result
≤ 36	Dengue Detected
≤ 37	Chikungunya Detected

Amplification Data



Sr. No.	Sample	C_t value
1.	Dengue Positive control	26.51
2.	Chikungunya Positive control	21.53
3.	Internal Control	27.91

Image representing amplification plot of Dengue and Chikungunya with Internal Control with Ct values using HiMedia's Hi-PCR® Dengue-Chikungunya Multiplex Probe PCR Kit. The results completely depend upon sample types.

Data Interpretation

Detection Channel			Result Interpretation
FAM (DENV)	HEX (CHKV)	Cy5 (Internal Control)	
+	-	+/-*	Positive for Dengue only
-	+	+/-*	Positive for Chikungunya only
+	+	+/-*	Positive for Dengue and Chikungunya
-	-	+	Negative for Dengue and Chikungunya
-	-	-	PCR inhibition or reagent failure. Repeat PCR or repeat extraction from original sample

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

Analytical Performance

Limit of Detection (LoD) - Analytical Sensitivity

Sensitivity for the HiMedia's Hi-PCR® Dengue-Chikungunya Multiplex Probe PCR Kit was conducted on InstaQ96® Real Time PCR system. The analytical sensitivity for the Hi-PCR® Dengue-Chikungunya Multiplex Probe PCR Kit was conducted using ATCC Synthetic RNA. The preliminary LoD of each target was determined by testing a 10-fold dilution series in triplicates per concentration, and then confirmed with 20 replicates of the concentration determined to be the LoD. The detectable limit of the HiMedia's Hi-PCR® Dengue-Chikungunya Multiplex Probe PCR Kit was determined to be 10 copies/µL for Dengue serotype 1 and 2; 5 copy/µL for Dengue serotype 3; 100 copies/µL for Dengue serotype 4, and 3 copies/µL for Chikungunya.

Inclusivity

In silico analysis for the assessment of inclusivity for the HiMedia's Hi-PCR® Dengue-Chikungunya Multiplex Probe PCR Kit was conducted by mapping the primers and probe against the available Dengue serotypes and Chikungunya sequences in GenBank. The HiMedia's Hi-PCR® Dengue-Chikungunya Multiplex Probe PCR Kit targets 100% of the known Dengue serotypes and Chikungunya strains.

Cross-reactivity - Analytical Specificity

In silico analysis was performed using NCBI nucleotide and Primer BLAST. The primers and probe for Dengue serotypes and Chikungunya were analyzed against organisms that are most frequently encountered in Tropical fever panel. Wet testing for analytical specificity was performed against the pathogens available in the laboratory as listed below.

Pathogens
Plasmodium falciparum
Salmonella typhi
Staphylococcus aureus
Enterococcus faecalis
Coronavirus (Covid-19)
Enterovirus
H1N1 influenza virus
Candida albicans

Warning

Not for Medicinal Use.

Precautions

Read the procedure carefully before starting the experiment. 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 Hi-PCR® Dengue-Chikungunya Multiplex Probe PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Quality Control

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

Troubleshooting Guide

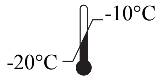
<table>
<tr><th>Sr. No.</th><th>Problem</th><th>Cause</th><th>Solution</th></tr>
<tr><td rowspan="3">1.</td><td rowspan="3">No amplification</td><td rowspan="2">Degraded samples</td><td>1. Check the integrity of RNA using agarose gel electrophoresis.</td></tr>
<tr><td>2. Use freshly prepared RNA to ensure the availability of intact template sequence for efficient amplification.</td></tr>
<tr><td>Error in protocol setup</td><td>Verify that the correct reagent volumes, dilutions and storage conditions have been used.</td></tr>
</table>

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-Chikungunya Multiplex 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.

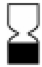
Technical Assistance
At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com.



Storage temperature

Do not use if package is damaged

HiMedia Laboratories Private Limited,
Reg. Off: Plot No. C-40, Road No. 21Y,
MIDC, Wagle Industrial Estate, Thane,
(West) 400604, Maharashtra, INDIA.
Web: www.himedialabs.com



01/2027

PIMBPCR179_O/0124 MBPCR179-09

Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory,diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.