MBPCR276 Hi-PCR® Dengue Serotyping-Chikungunya-Zika Multiplex Probe PCR Kit

Description

Arboviral infections such as Dengue, Chikungunya and Zika are transmitted to humans through bites of infected mosquitoes specifically *Aedes aegypti* and *Aedes albopictus*. In the recent years, dengue (DENV), chikungunya (CHIKV) and Zika (ZIKV) are extending their geographical range from tropical and subtropical zones to temperate zones. Similarities in clinical manifestations and overlapping symptoms between these diseases impose a challenge in differential diagnosis. Although not very common, there are reported cases of co-circulation of multiple DENV serotypes and co-infection with all the three viruses in endemic regions. The above reasons underscore the importance of differential diagnosis between DENV, CHIKV and ZIKV. Hi-PCR® Dengue Serotyping-Chikungunya-Zika Multiplex Probe PCR Kit allows fast, reliable solution for Dengue serotyping and detection of Chikungunya and Zika in a single assay.

NOTE: Hi-PCR® Dengue Serotyping-Chikungunya-Zika Multiplex Probe PCR Kit is for *in-vitro* use only.

Intended Use: Hi-PCR® Dengue Serotyping-Chikungunya-Zika Multiplex Probe PCR Kit is designed for typing of Dengue virus serotypes 1-4 and detection of Chikungunya and Zika viruses in clinical samples.

Principle

Hi-PCR® Dengue Serotyping-Chikungunya-Zika Multiplex Probe PCR Kit is based on real-time PCR technology employing reverse transcription of RNA template and subsequent pathogen 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-Chikungunya-Zika Multiplex Probe PCR Kit allows simultaneous typing of Dengue serotypes DENV-1, DENV-2, DENV-3 and DENV-4 and detection of Chikungunya and Zika viruses 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, CHIKV, ZIKV RNA labelled with the FAM, HEX and Texas Red fluorophore respectively. In addition, tube 2 contains an endogenous internal control amplification system (probe labelled with fluorophore Cy5) to verify efficient 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, Molecular Biology grade water is used as the template. It is recommended to have a minimum of one reaction of no template control per run.

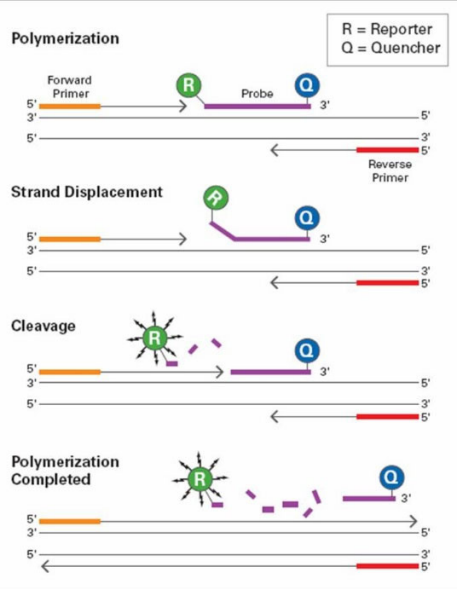
ISO 9001-2015 CERTIFIED WHO GMP CERTIFIED ISO 13485-2016 CERTIFIED

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

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.

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

- Simultaneous serotyping of Dengue viruses and detection of Chikungunya and Zika in a single assay
- 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
- High sensitivity and specificity
- Suitable for 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

Sample Type: RNA extracted from serum or EDTA plasma of human origin

Storage and Shelf life

The provided kit has a shelf-life of 12 months when stored between -10°C and -20°C. Repeated thawing and freezing of PCR reagents should be avoided, not more than 3 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 and thaw. 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 (µL)	50R (µL)	100R (µL)
DENV1-CHIK-ZIK Master Mix	DS1682	324	648	1272
DENV2-4 Primer Probe Mix (Tube 1)	DS1676	81	162	318
DENV1-CHIK-ZIK-IC Primer Probe Mix (Tube 2)	DS1677	81	162	318
Molecular Biology Grade Water for PCR	ML065	486	972	1908
Positive Control Tube 1	DS1680	81	162	318
Positive Control Tube 2	DS1681	81	162	318

***** For a 30 µL PCR reaction

Materials needed but not provided

All 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-Chikungunya-Zika Multiplex Probe PCR Kit contains fluorophores that are compatible to the following 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 /	Roche	FAM	JOE/HEX/VIC	ROX/Texas Red	Cy5
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 manufactureŕs instructions and recommendations

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 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.

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			
DENV1-CHIK-ZIK Master Mix	DS1682	6 µL	6 µL
DENV2-4 Primer Probe Mix (Tube 1)	DS1676	3 µL	----
DENV1-CHIK-ZIK-IC Primer Probe Mix (Tube 2)	DS1677	----	3 µL
Molecular Biology Grade Water for PCR	ML065	6 µL	6 µL
Total PCR Reaction Mix	-	15 µL	15 µL
Template addition			
Template/ Purified 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 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			
Positive Control 1	DS1680	15 µL	-
Positive Control 2	DS1681	-	15 µL
No Template Control			
Molecular Biology (MB) Grade Water for PCR		15 µL	15 µL
Total reaction volume	-	30 µL	30 µL

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	FAM	None
	CHIKV	HEX	None
	ZIKV	Texas Red	None
	Internal Control (IC)	Cy5	None

Please select ‘Passive reference dye’ as ‘None’ wherever applicable

Data Analysis

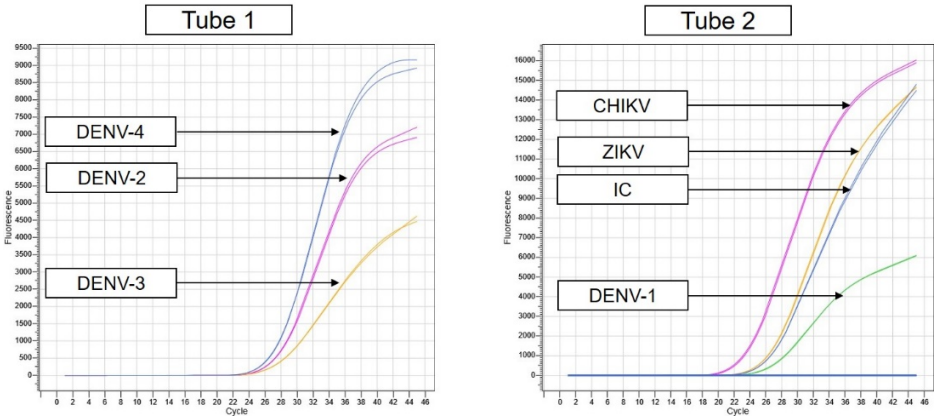
The following conditions should be met for a valid diagnostic test:

Control	Detection channel						
	TUBE 1			TUBE 2			
	FAM (DENV-2)	Texas Red (DENV-3)	Cy5 (DENV-4)	FAM (DENV-1)	HEX (CHIKV)	Texas Red (ZIKV)	Cy5 (IC)
Positive Control	+	+	+	+	+	+	+
No template Control	-	-	-	-	-	-	-

NOTE: For BioRad CFX Opus 96, 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 (+)
CHIKV	≤ 40	Detected (+)
ZIKV	≤ 40	Detected (+)

Amplification plot:



Sr. No	Tube-1	Ct value	
		PTC	NTC
1	DENV-2	28.60	--
2	DENV-3	28.14	--
3	DENV-4	27.97	--

Sr. No	Tube-2	Ct value	
		PTC	NTC
1	DENV-1	27.23	--
2	CHIKV	24.17	--
3	ZIKV	26.95	--
4	IC	26.88	--

Note: Image representing probe based Real-Time amplification of Dengue-1, 2, 3,4, Chikungunya, Zika and IC (Ct values provided in table are for representation).

Data Interpretation:

For single virus infection							
Detection Channel (Target)							Result Interpretation
Tube 1			Tube 2				
FAM (DENV-2)	Texas Red (DENV-3)	Cy5 (DENV-4)	FAM (DENV-1)	HEX (CHIKV)	Texas Red (ZIKV)	Cy5 (IC)	
+	-	-	-	-	-	+	Positive for DENV-2**#
-	+	-	-	-	-	+	Positive for DENV-3**#
-	-	+	-	-	-	+	Positive for DENV-4**#
-	-	-	+	-	-	+*	Positive for DENV-1**#
-	-	-	-	+	-	+*	Positive for CHIKV#
-	-	-	-	-	+	+*	Positive for ZIKV#
-	-	-	-	-	-	+	Negative for DENV 1-4, CHIKV and ZIKV
-	-	-	-	-	-	-	Inconclusive test*** Likely poor extraction or sample quality. PCR inhibition or reagent failure.

*The presence or absence of a signal in the Cy5 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 or more 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".
# If sample is positive for DENV serotypes and/or Chikungunya and/or ZIKV, re-test the specimen. If sample is repetitively positive for both the viruses, the result may be indicative of a "co-infection".
*** 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: 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-Chikungunya-Zika Multiplex Probe PCR Kit 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, DENV-4 and Chikungunya and Quantitative Genomic RNA from Zika virus. The detectable limit of the Hi-PCR® Dengue Serotyping-Chikungunya-Zika Multiplex Probe PCR Kit was determined to be 2.87 copies/µL for DENV-1, < 1 copy/µL for DENV-2, 1.87 copies/µL for DENV-3, 1.41 copies/µL for DENV-4, < 1 copy/µL for CHIKV and < 1 copy/µL for ZIKV.

Inclusivity - Analytical Specificity

The analytical specificity of the Hi-PCR® Dengue Serotyping-Chikungunya-Zika Multiplex Probe PCR Kit 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, DENV-4, CHIKV and ZIKV available in the GenBank database.

Analytical Reactivity

The analytical reactivity of the Hi-PCR® Dengue Serotyping-Chikungunya-Zika Multiplex Probe PCR Kit was verified by wet lab testing of the oligonucleotides (primers and probes) against commercially available controls or standards - AMPLIRUN® DENGUE VIRUS RNA CONTROLS from Vircell Microbiologics [DENV 1 (KM204119.1) and DENV 2 (KM204118.1)] and Quantitative Genomic RNA from Zika virus strain PRVABC59 (ATCCVR-1843DQ).

Cross-Reactivity and Interference with Other Microorganisms

Wet testing analysis was performed against the DNA/RNA of the pathogens available in the laboratory on Bio-Rad CFX Opus 96 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-Chikungunya-Zika Multiplex Probe PCR Kit.

Human coronavirus 229E	Human adenovirus 1 strain Adenoid 71
Influenza A virus (H1N1) pdm09 strain A	*Plasmodium falciparum* strain 3D7
Influenza A virus (H3N2) strain A	*Plasmodium vivax* (Grassi and Feletti) Labbe
Influenza B virus (Yamagata Lineage)	*Leptospira interrogans*
Hepatitis B virus	Human metapneumovirus
Hepatitis C virus	Human herpesvirus 1 MacIntyre (HSV-1)
Hepatitis A virus	Human herpesvirus 2 (HSV-2)
Hepatitis E virus	Epstein-Barr virus
Human immunodeficiency virus 1 (HIV-1)	*Candida albicans*
Middle East respiratory syndrome coronavirus	*Salmonella typhi*

Cross-Reactivity Analysis – *in silico*

The sequences of the oligonucleotides (primers and probes) in the Hi-PCR® Dengue Serotyping-Chikungunya-Zika Multiplex Probe PCR Kit 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)	*Brucella melitensis* (taxid:29459)
Yellow fever virus (taxid:11089)	*Borrelia burgdorferi* (taxid:139)
Yellow fever virus group (taxid:40005)	*Orientia tsutsugamushi* (taxid:784)
Japanese encephalitis virus (taxid:11072)	*Salmonella* (taxid:590)
Japanese encephalitis virus group (taxid:11071)	*Corynebacterium diphtheriae* (taxid:1717)
Spondweni virus (taxid:64318)	*Burkholderia* sp. (taxid:36773)
Spondweni virus group (taxid:297696)	*Leptospira* (taxid:171)
Tick-borne encephalitis virus (taxid:11084)	*Plasmodium* (taxid:5820)
Tick-borne encephalitis virus group (taxid:29263)	Hepatitis B virus (taxid:10407)

Modoc virus (taxid:64300)	Hepatitis C virus (taxid:11103)
Tamana bat virus (taxid:161675)	Hepatitis E virus (taxid:291484)
Cell fusing agent virus (taxid:31658)	Human hepatitis A virus (taxid:208726)
Omsk hemorrhagic fever virus (taxid:12542)	Human immunodeficiency virus (taxid:11676)
St. Louis encephalitis virus (taxid:11080)	Cytomegalovirus (taxid:10358)
Murray Valley encephalitis virus (taxid:11079)	Epstein-Barr virus (taxid:10376)
Kyasanur Forest disease virus (taxid:33743)	Human adenovirus sp. (taxid:1907210)
Rift Valley fever virus (taxid:11588)	Human parvovirus B19 (taxid:10798)
Israel turkey meningoencephalomyelitis virus (taxid:64291)	Monkeypox virus (taxid:10244)
Ntaya virus (taxid:64292)	Wesselsbron virus (taxid:164416)
Tembusu virus (taxid:64293)	Measles morbillivirus (taxid:11234)
Sepik virus (taxid:44026)	Mumps orthorubulavirus (taxid:2560602)
Usutu virus (taxid:64286)	Rubella virus (taxid:11041)
Aroa virus (taxid:64303)	*Rickettsia* sp. (taxid:789)
Bussuquara virus (taxid:64304)	*Mycobacterium tuberculosis* (taxid:1773)
Iguape virus (taxid:64308)	*Klebsiella pneumoniae* (taxid:573)
Naranjal virus (taxid:64313)	*Coxiella burnetii* (taxid:777)
Cacipacore virus (taxid:64305)	*Staphylococcus aureus* (taxid:1280)
Koutango virus (taxid:44025)	*Streptococcus pneumoniae* (taxid:1313)
Alfuy virus (taxid:44017)	*Pseudomonas aeruginosa* (taxid:287)
Kunjin virus (taxid:11077)	Varicella Zoster Virus (taxid:10335)
Yaounde virus (taxid:64319)	*Burkholderia pseudomallei* (taxid:28450)
Kokobera virus (taxid:44024)	*Leishmania donovani* (taxid:5661)
Stratford virus (taxid:44027)	agent of lymphatic filariasis (taxid:6279)
Bagaza virus (taxid:64290)	Ebola virus (taxid:1570291)
Ilheus virus (taxid:59563)	Hantavirus (taxid:1980442)
Rocio virus (taxid:64315)	Enterovirus (taxid:12059)
Lymphocytic choriomeningitis virus (taxid:3052303)	*Bacillus anthracis* (taxid:1392)
Salmonella paratyphi (taxid:54388)	*Neisseria meningitidis* (taxid:487)
Aspergillus (taxid:5052)	*Yersinia pestis* (taxid:632)
Histoplasma (taxid:5036)	*Acinetobacter baumannii* (taxid:470)
Streptococcus pyogenes (taxid:1314)	*Listeria monocytogenes* (taxid:1639)

Evaluation

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

Quality Control

Each lot of Hi-PCR® Dengue Serotyping-Chikungunya-Zika Multiplex Probe PCR Kit is assayed for contaminating endonuclease, exonuclease, and non-specific DNase activities. It has been functionally tested in amplification assay.

Troubleshooting Guide

Sr. No.	Problem	Cause	Solution
1.	No amplification	Degraded samples	Check the integrity of nucleic acid using agarose gel electrophoresis.
			Use freshly prepared RNA to ensure the availability of intact template sequence for efficient amplification.
		Error in protocol setup	Check whether all components are added in correct volume as per the manual.
		Inappropriate storage conditions	Store the reagents at recommended temperature for its optimal performance. Check expiry of the reagents and use new lot of reagents if necessary.
		Incorrect PCR programming	Ensure selection of appropriate fluorescence channel as detailed in the manual. Compare the PCR program to the manual.
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. Use calibrated pipettes. Repeat the run.
3.	Amplification in No template control	Cross contamination during handling	Replace all critical solutions. Repeat the analysis of all tests with fresh aliquots of critical reagents. Follow good laboratory practices to avoid contamination issues.

Safety Information

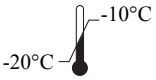
Hi-PCR® Dengue Serotyping-Chikungunya-Zika Multiplex Probe PCR Kit for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

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.

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

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