

## MBPCR112 Chikungunya Detection Kit (Real-Time Probe Based PCR)

### Instructions For Use

#### Description

Chikungunya virus (CHIKV) is a viral disease transmitted to humans by infected mosquitoes. It is an RNA virus with a positive-sense single-stranded genome of about 11.6kb and belongs to the alphavirus genus of the family Togaviridae. The virus is transmitted from human to human by the bites of infected female mosquitoes. Most commonly, the mosquitoes involved are *Aedes aegypti* and *Aedes albopictus*, two species which can also transmit other mosquito-borne viruses, including dengue. These mosquitoes can be found biting throughout daylight hours, though there may be peaks of activity in the early morning and late afternoon. There is no specific antiviral drug treatment for CHIKV. Treatment is directed primarily at relieving the symptoms, including joint pain using anti-pyretics, optimal analgesics and fluids. There is no commercial chikungunya vaccine. Nucleic acid amplification-based assays or Polymerase Chain Reaction (PCR) is an alternative method that allows for sensitive and specific detection of Chikungunya from clinical samples. 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 and non-infectious pathogens and genes.

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

#### Intended Use

Recommended for sensitive and specific detection of 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 Chikungunya Detection Kit (Real-Time Probe Based PCR) is designed to detect the **Non-structural gene of CHIKV in FAM channel** and **Internal Control in HEX channel** in a single tube reaction. The kit allows sensitive and specific detection of CHIKV in a single tube reaction.

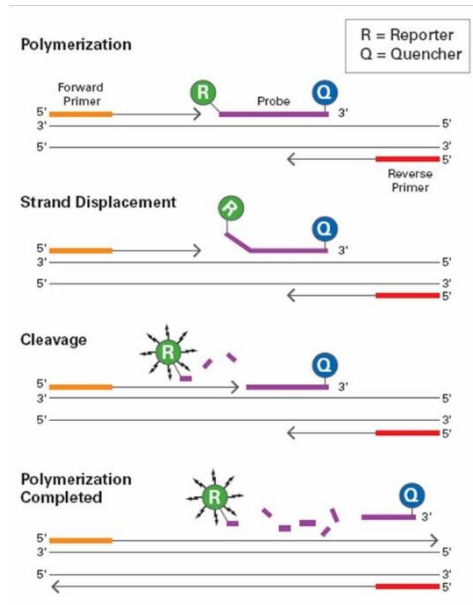
#### Positive control

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

#### Internal Control

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

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



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

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

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

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

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

### Molecular Features

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

### Technology features:

- Fast and reliable results within 90 minutes.
- One-step assay i.e. reverse transcription and amplification are performed in the 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.

**Sample types:** Serum / Blood sample / Viral cultures

### Storage and Shelf life

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

### 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 to in individual safety data sheets.

**Kit Contents:**

The provided PCR kit contains:

Components	Product Code	Reagents provided for (reactions)*	
		25R	50R
RT Buffer	DS0221	135	270
10X solution H	DS0222	68	135
M-MuLV Reverse Transcriptase	DS0220	27	54
Primer Mix for Chikungunya	DS0184	54	108
Chikungunya Probe	DS0212	34	68
Primer Mix for Internal Amplification Control (IAC)	DS0124	27	54
Internal Amplification Control (IAC) Probe	DS0214	34	68
Chikungunya Positive control	DS0213	5	10
Molecular Biology Grade Water for PCR	ML065	200	350

\* For a 25 µL PCR reaction

**Sample Preparation**

Various clinical samples / cultured isolates are routinely examined. For extraction and purification of pure RNA for high yield, perform the nucleic acid purification using HiPurA® Viral RNA Purification Kit (MB615) as instructed in the protocol.

**Materials needed but not provided**

Appropriate real-time PCR instrument

Appropriate nucleic acid extraction system or kit

Centrifuge with a rotor for 1.5 mL - 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](http://www.himedialabs.com)

Product name	Product Code
<b>Real-Time PCR Instrument and equipment</b>	
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
<b>Automated nucleic acid extraction system and materials</b>	
Insta NX® Instrument - fully automated nucleic acid purification system utilizing the Innovative Super -S membrane column method	LA1056
Insta NX® Mag16, Insta NX® Mag16 <sup>Plus</sup>	LA1118, MBLA018
Insta NX® Mag32, Insta NX® Mag32 <sup>Plus</sup>	LA1096, MBLA019
Insta NX® Mag96	LA1097

<b>Extraction Kits</b>	
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
<b>Tubes, plates and other consumables</b>	
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**

HiMedia's Chikungunya Detection Kit (Real-Time Probe Based PCR) contains fluorophores that are compatible to the following PCR systems. HiMedia's Chikungunya Detection Kit (Real-Time Probe Based PCR) however has been validated on Bio-Rad CFX Opus 96, Applied Biosystems QuantStudio 5 and Insta Q96® Plus Real Time PCR Systems.

<b>Real-Time PCR system</b>	<b>Company</b>	<b>Dye 1</b>	<b>Dye 2</b>
Insta Q96® AG/ Insta Q96® AG 6.0/Insta Q96® - 6.0/Insta Q96® Plus/Insta Q48® M4	HiMedia Laboratories Pvt. Ltd.	FAM	HEX
BioRad CFX Opus 96/CFX96 Touch/ CFX384 Touch	Bio-Rad Laboratories, Inc.	FAM	JOE/HEX
QuantStudio™ 5	Applied Biosystems	FAM	JOE/HEX/VIC
ABI® Prism SDS 7500	Applied Biosystems	FAM	JOE/HEX/VIC
QIAquant 96 & 384 5plex	QIAGEN	FAM	JOE/HEX
Rotor-Gene®6000 & Q	QIAGEN	FAM	JOE/HEX
LightCycler® 96 / LightCycler® 480	Roche	FAM	JOE/HEX/VIC
qTOWER <sup>3</sup>	Analytik Jena	FAM	JOE/HEX/VIC

**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 Master 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.

- Based on the number of specimens to be tested (N), calculate the volume of the components to be added as N\* volume of "1X"
- Use 1.5 mL Nuclease free centrifuge tube(s) for the preparation of the PCR reaction mix of Tube 1 and Tube 2. Refer to 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)
RT Buffer	DS0221	5 µL
10X solution H	DS0222	2.5 µL
M-MuLV Reverse Transcriptase	DS0220	1 µL
Primer Mix for Chikungunya	DS0184	2 µL
Chikungunya Probe	DS0212	1.25 µL
Primer Mix for Internal Amplification Control (IAC)	DS0124	1 µL
Internal Amplification Control (IAC) Probe	DS0214	1.25 µL
RNA template	-	5 µL
Molecular Biology Grade Water for PCR	ML065	Upto 25 µL

**NOTE: The user can also set up an additional PCR reaction containing 1 µL of Positive control RNA (provided) in a separate tube.**

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

#### Recommended PCR program

- |                          |                                    |                     |
|--------------------------|------------------------------------|---------------------|
| 1. Reverse Transcription | : 50°C for 15 minutes              | } No. of cycles: 40 |
| 2. Initial Denaturation  | : 95°C for 2 minutes 30 seconds    |                     |
| 3. Denaturation          | : 94°C for 30 seconds              |                     |
| 4. Annealing             | : 60°C for 30 seconds (Plate Read) |                     |
| 5. Extension             | : 72°C for 30 seconds              |                     |
| 6. Final Extension       | : 72°C for 5 minutes               |                     |

#### Data Analysis

##### Selection of channels

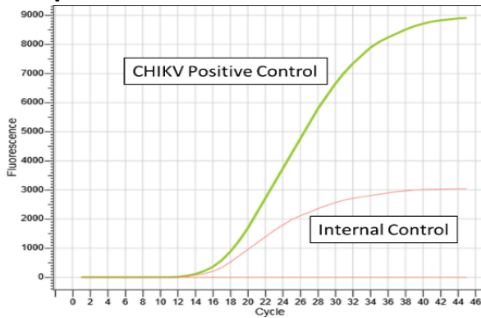
Target	Channels	Quencher
Chikungunya	FAM	None
Internal Control	HEX/JOE/VIC	None

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

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

Control	Detection channel	
	Chikungunya (FAM)	Internal Control (HEX)
<b>Positive Control</b>	+	+
<b>Negative Control</b>	-	+

### Amplification Data



Sr. No.	Sample	C <sub>t</sub> value
1.	Chikungunya positive control	13.89
2.	Internal Control	15.8

Image representing amplification plot of Chikungunya virus RNA with Ct values using HiMedia's Chikungunya Detection Kit (Real-Time Probe Based PCR). The results completely depend upon sample types.

### Data Interpretation:

Ct value	Result
≤ 35	Detected (+)
> 35 or N/A	Not detected (-)

Detection Channel		
Chikungunya (FAM)	Internal Control (HEX)	Result Interpretation
+	±	Positive for Chikungunya
-	+	Negative for Chikungunya
-	-	PCR inhibition or reagent failure. Repeat PCR or repeat extraction from original sample

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

### 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 HiMedia's Chikungunya Detection Kit (Real-Time Probe Based PCR) 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 Chikungunya. The detectable limit of HiMedia's Chikungunya Detection Kit (Real-Time Probe Based PCR) was determined to be 1 copies/µL.

### Inclusivity

*In silico* analysis for the assessment of inclusivity for the HiMedia's Chikungunya Detection Kit (Real-Time Probe Based PCR) was conducted by mapping the primers and probe against the available sequences in GenBank. The HiMedia's Chikungunya Detection Kit (Real-Time Probe Based PCR) targets 100% of the characterized strains.

### Warning

Certified for *in vitro* Diagnostic Use (IVD). Not for Medicinal Use.

### 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 the 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 Chikungunya Detection Kit (Real-Time Probe Based PCR) is tested against predetermined specifications to ensure consistent product quality. The kit provides Chikungunya positive control to be included in every run.

### 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.
		Error in reaction set-up	Prepare a large volume master mix, vortex thoroughly and aliquot into reaction tubes.
2.	Variability between replicates	Air bubbles in reaction mix	Briefly centrifuge reaction samples/plate prior to running on a real-time PCR instrument.
		Pipetting error	C <sub>t</sub> values of replicates can show increased variation due to poor laboratory technique or imprecise pipettes.
		Reagents contaminated	1. Replace all critical solutions. 2. Repeat the analysis of all tests with fresh aliquots of critical reagents.
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.
5.	Weak or no signal of the IAC		

### Safety Information

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




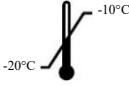







### Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Following 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](mailto: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 :**

	<b>AR Experts B.V.</b> Boeingavenue 209, 1119 PD, Schiphol-Rijk, The Netherlands
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**Disclaimer :**

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