MBPCR102 Hi-PCR® Generic Dengue Semi-Q PCR Kit

Description

Dengue fever is a mosquito-borne tropical disease caused by the dengue virus. Dengue is spread by several species of mosquito of the *Aedes* type, principally *A. aegypti*. The virus has five different types; infection with one type usually gives lifelong immunity to that type, but only short-term immunity to the others. Subsequent infection with a different type increases the risk of severe complications. Dengue fever virus (DENV) is an RNA virus of the family *Flaviviridae*; genus *Flavivirus*. There are five strains of the virus, called serotypes, of which the first four are referred to as DENV-1, DENV-2, DENV-3 and DENV-4. With healthcare becoming increasingly available to a large number of people, specific and faster methods for pathogen detection are the need of an hour.

The **Hi-PCR® Generic Dengue Semi-Q PCR Kit** is designed to detect the specific gene regions of **D1 & D2 gene (511 bp)** as generic primer. These unique sequences are found exclusively within the members of the Dengue, and because of this exclusivity, it has become an important diagnostic tool in the identification of Dengue species. The kit allows rapid, sensitive and specific detection of Dengue samples.

NOTE: HiMedia's Hi-PCR® Generic Dengue Semi-Q PCR Kit is for *in-vitro* use only.

Intended Use

The kit is designed for *in vitro* diagnostics and provides qualitative detection. This diagnostic kit assures sensitive detection in clinical samples.

Principle:

HiMedia's Hi-PCR® Generic Dengue Semi-Q PCR Kit is a qualitative conventional PCR kit which includes the amplification of **D1 & D2 (511 bp)** gene for Dengue Generic. The amplified target is confirmed by using agarose gel electrophoresis.

One-Step Semi Quantitative Polymerase Chain Reaction (PCR) is a very sensitive and specific method for amplification based detection of genes. The four steps of a successful PCR reaction in One-Step Semi Quantitative PCR which include **Reverse transcription step** that help conversion of RNA to cDNA, Denaturation, Annealing and Extension. The double-stranded DNA melts and forms single stranded DNA at high temperature (Denaturation). Sequence-specific primers bind to the target sequence on single-stranded DNA at lower temperature (Annealing). Taq DNA Polymerase adds dNTPs onto the single stranded DNA at intermediate temperature (Extension). These 3 steps of PCR are usually repeated between 25 to 40 times in each PCR assay.

Gel electrophoresis is used to analyze the amplification of desired gene region based on separation of DNA fragments according to their size.

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

Features:

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

Sample Source: Blood, Plasma & Serum samples from suspected cases

Storage:

The provided kit has a shelf-life of 12 months when stored between -10°C to -20°C. Repeated thawing and freezing of PCR reagents should be avoided as this may reduce the sensitivity. If reagents are to be used multiple times, we recommend storing reagents as aliquots to avoid repeated freeze and thaw. Degradation of sample DNA specimens can also reduce sensitivity of the assay. HiMedia does not recommend using the kit after the expiry date stated on pack.

Kit contents

The provided PCR kit contains:

Components	Product codes	Reagents provided for (reactions)* (µL)	
		25R	**50R**
RT Buffer	DS0221	270	540
10X solution H	DS0222	135	270
M-MuLV Reverse Transcriptase	DS0220	54	108
Primer mix for Generic Dengue (SYBr)	DS0369	54	108
Generic Dengue Positive Control	DS0215	25	50
Molecular Biology Grade Water for PCR	ML065	700	1300
100 bp DNA ladder	MBT049	20	40
6X Gel Loading Buffer	ML015	54	108

*For 50 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:

For preparation of Viral RNA, perform the nucleic acid purification using HiMedia's HiPurA® Viral RNA Purification Kit (MB615) as described 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)
- Thermal Cycler (Product Code: LA948/LA949/LA950/LA1006/LA1015/LA1059/LA1060/LA1066)
- Barrier Micropipette Tips (Product Code: LA749 / LA749A / LA751 / LA751A / LA750 / LA750A / LA859 / LA859A)

- Micropipettes

General Preparation Instructions:

- Before use, suitable amount of all PCR components should be completely thawed on ice (4°C).
- Perform the amplification reactions in a clean area.
- Use of aerosol barrier pipette tips is recommended to reduce contamination risks from extraneous DNA templates.
- Extract and store positive control material (if used) separately from all other reagents to avoid contamination and add it to the reaction mix in a separate area.
- Centrifuge the components briefly once thawed.

A. Protocol for PCR Master Mix Preparation

Perform PCR reaction for each RNA sample as per the following table:

Components	Recommended volume to be added per reaction (µL)
RT Buffer **(DS0221)**	10 µL
10X Solution H **(DS0222)**	5 µL
M-MuLV Reverse Transcriptase **(DS0220)**	2 µL
Primer Mix for Generic Dengue **(DS0369)**	2 µL
Template (Extracted RNA)	Upto 5 µL
Molecular Biology Grade Water for PCR **(ML065)**	Upto 50 µl

NOTE: (Optional) – The user can also set up an additional PCR reaction containing 5µL of Positive control RNA (provided) in a separate tube.

Centrifuge the tube briefly at 6000 rpm for about 10 seconds and place the tubes in the PCR machine and set the recommended PCR program (mentioned below). Interpret the data using Agarose Gel Electrophoresis.

B. Recommended PCR program:

1	Reverse Transcription	50°C for 15 minutes	No. of cycles: 1
2	Initial denaturation	95°C for 2 minutes 30 seconds	No. of cycles: 1
3	Denaturation	94°C for 30 seconds	No. of cycles: 35
4	Annealing	55°C for 30 seconds	
5	Extension	72°C for 30 seconds	
6	Final Extension	72°C for 5 minutes	No. of cycles: 1

C. After amplification, the products can be kept at 4°C overnight or frozen at –20°C for long-term storage.

D. PCR Assay Results Interpretation:

- For analysis of the PCR data, load 10 µl of amplicon on a 1.5% Agarose gel along with 1 µl of 6X Gel Loading Buffer (ML015).
- Load 4 µl of 100 bp DNA ladder (MBT049) in separate well.

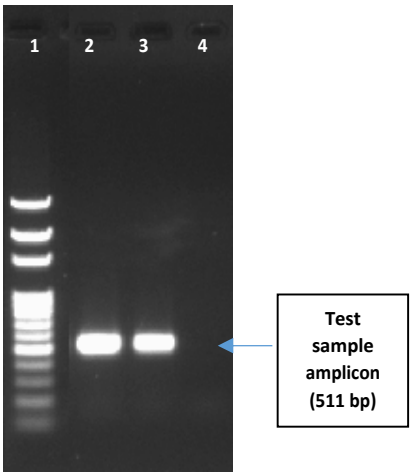
E. EtBr-staining to check results:

- Incorporate EtBr in the agarose gel or stain the agarose gel with EtBr for 10-15 min.
- Confirm the expected amplicon size comparing with 100 bp DNA marker.

Quality Control:

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

Amplification Data:



Lane no.	Samples
1	100 bp ladder
2	Amplicon of Dengue Sample (511 bp)
3	
4	Negative control

Image representing One-Step Semi Quantitative PCR Data of Generic Dengue samples (511 bp)

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 in safety data sheets of the product.

Performance and Evaluation

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

Troubleshooting Guide

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

2.	Variability between replicates	Error in reaction setup	Prepare large volume reaction mix, vortex thoroughly and aliquot appropriately into reaction tubes.
		Air bubbles in reaction mix	Briefly centrifuge reaction samples/plate prior to running on a PCR machine.
		Pipetting error	Replicates can show increased variation due to poor laboratory techniques 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.

Safety Information

The Hi-PCR® Generic Dengue Semi-Q 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 off 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.

IVD	In vitro diagnostic medical device
CE	CE Marking
-10°C -20°C	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
EC REP	CE Partner 4U ,Esdoornlaan 13, 3951 DB Maarn The Netherlands, www.cepartner 4u.eu
	01/2027

PIMBPCR102_O/0124 MBPCR102-09

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.