MBPCR221 Hi-PCR® Hepatitis E Virus Probe PCR Kit

Description

Hepatitis E Virus (HEV) is a single-stranded, positive-sense RNA virus with a linear genome approximately 7.5 kB in length, classified under the Hepeviridae family. It is the causative agent of Hepatitis E, an inflammatory liver disease. HEV primarily spreads through the fecal-oral route, often due to consumption of contaminated water. The virus has an incubation period of 3 to 8 weeks and can cause symptoms such as nausea, loss of appetite, abdominal pain, and jaundice. In developing regions of Asia, Africa, and Latin America, HEV is a major contributor to acute viral hepatitis, accounting for up to 50% of cases. Seroprevalence studies in endemic regions show high infection rates, ranging from 15% to 60%. Although serological methods for detecting HEV in environmental and food samples have been challenging, real-time RT-PCR, following RNA extraction and purification, remains the preferred method due to its rapidity and sensitivity. Serological methods have proven inadequate for detecting Hepatitis E Virus (HEV) in environmental or food samples, and cell culture-based detection is challenging. Real-time RT-PCR, after extraction and purification of the viral RNA, is the preferred method for HEV detection due to its speed and high sensitivity.

NOTE: HiMedia's Hi-PCR® Hepatitis E Virus Probe PCR Kit is for *in-vitro* use only.

Intended Use

Hi-PCR® Hepatitis E Virus (HEV) Probe PCR Kit is intended for use by qualified clinical laboratory personnel trained in the techniques of real-time PCR and in vitro diagnostic procedures. The kit is recommended for sensitive and specific detection of HEV RNA in water samples. The Hi-PCR® Hepatitis E Virus (HEV) probe PCR kit targets 100% of the known HEV genotypes.

Product Description:

Hi-PCR® Hepatitis E Virus (HEV) Probe PCR Kit is based on real-time PCR technology for the detection of Hepatitis E Virus (HEV) specific RNA. The kit contains primer-probe mixture specific for the detection of HEV RNA. In addition, the kit contains a positive control and internal control for identification of possible PCR inhibition or reagent failure. The assay principle is based on the hydrolysis probe chemistry which confers higher specificity and sensitivity.

Positive control

This is a control reaction using a known template (target pathogen). A positive control is usually used to ensure proper and intended functioning of all the PCR reagents and is recommended to be included in every run to assess optimal performance.

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 one reaction of negative control per run.

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

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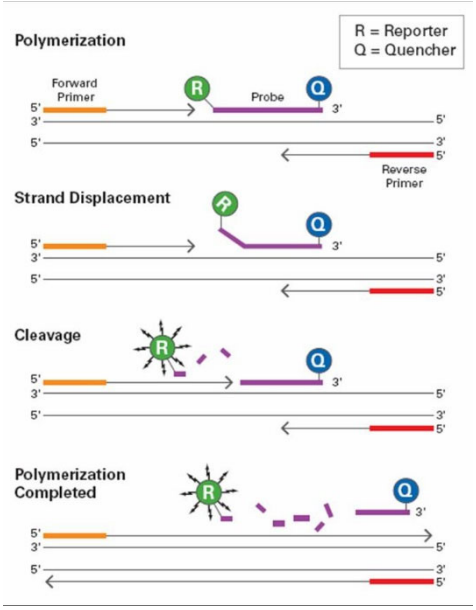
oligonucleotides having a fluorescent reporter dye attached to the 5' end and a quencher dye to the 3' end. HiMedia's Hi-PCR® Hepatitis E Virus (HEV) Probe PCR Kit is designed to specifically detect HEV viral RNA in FAM channel and Internal Control (IC) in JOE channel in a single tube reaction.

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.

Molecular and Technology Features

- Fast and reliable results within 90 minutes.
- Includes all reagents and controls for the validity of the test.
- Open system – Compatible with any 3-channel, 4-channel and 5-channel qPCR cyclers.
- Good sensitivity and specificity.
- 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 Specimen: Water sample

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 blood. Safety guidelines may be referred in individual safety data sheets.

Storage and Shelf life

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 (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 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.

Kit Contents: The provided PCR kit contains

Components	Product code	Reagents provided for (reactions)* (µL)	
		25R	50R
2X One-step Buffer Mix	DS1086	270	540
One-step RT enzyme Mix	DS1087	22	44
HEV Primer-Probe Mix	DS1826	32	64
HEV Positive Control	DS1827	43	86
Molecular Biology Grade Water for PCR	ML065	43	86

***** For a 20µL 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).

Materials needed but not provided

All materials are available through www.himedialabs.com

Product name	Product Code
Real-Time PCR Instruments and other 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
HiPer® Mini Plate Centrifuge	LA1099
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
Extraction Kits	
HiPurA® Water Nucleic Acid Purification Kit	MB622

HiPurA® Water Viral RNA Purification Kit (with Nitrocellulose Membrane Filter)	MB620A
HiPurA® Water Viral RNA Purification Kit	MB620
HiPurA® Pre-filled plates for Water Nucleic Acid Purification Kit	MB622MPF16
HiPurA® Pre-filled Cartridges for Water nucleic Acid Purification (Insta NX® Mag16)	MB622PC16
HiPurA® Pre-filled plates for Water Nucleic Acid Purification Kit (Insta NX® Mag32)	MB622MPF-32
HiPurA® Pre-filled plates for Waste Water Nucleic Acid Purification Kit	MB622MPF-32A
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,100µl Max capacity 100 µL	LA1104A
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, 0.1 mL, 0.2 mL; PCR Plates	PW1255/PR2/PR3/PR19
Optical Sealing film	PR18
1.5 ml nuclease free Micro centrifuge tubes	PW146

Kit compatibility with Real-Time PCR Systems

Hi-PCR® Hepatitis E Virus (HEV) Probe PCR Kit contains fluorophores that are compatible to the following PCR systems:

Real-Time PCR system	Company	Dye 1 (HEV)	Dye 2 (IC)
Insta Q96®AG/ Insta Q96®AG 6.0/Insta Q96® - 6.0/Insta Q96® Plus/Insta Q48® M4	HiMedia Laboratories Pvt. Ltd.	FAM	JOE
QuantStudio™ 3 and 5 / Quant StudioTM 6 and 7 Flex Real-Time PCR Systems / QuantStudioTM Dx	Applied Biosystems	FAM	JOE
Applied Biosystems 7500	Applied Biosystems	FAM	JOE
BioRad CFX Opus 96/CFX96	Bio-Rad Laboratories, Inc.	FAM	JOE
Rotor-Gene® Q/QIAquant	QIAGEN	Green	Yellow
Roche LightCycler® 96	Roche	FAM	JOE
AriaMx	Agilent	FAM	JOE
Alta RT-96/48	Athenese-Dx Private Limited	FAM	JOE
qTOWER^3 auto	Analytik Jena	FAM	JOE

Note: Ensure that the Real-Time PCR system is calibrated for dyes and is maintained according to the manufactureŕs 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/RNA 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.
- Clear surfaces and working areas with RNA Kil™ (ML162).

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 X (volume of "1X")
3. Use 1.5 mL Nuclease free centrifuge tube(s) for the preparation of the PCR reaction mix. 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)
Preparation of PCR Reaction Mix		
2X One-step Buffer Mix	DS1086	10.0 µL
One-step RT enzyme Mix	DS1087	0.8 µL
HEV Primer-Probe Mix	DS1826	1.2 µL
Total PCR Reaction Mix	**-**	**12.0 µL**
Template addition		
Purified Viral RNA	-	8.0 µL
Total reaction volume	**-**	**20.0 µL**

4. Aliquot 12.0 µL of 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 8.0 µL of extracted RNA of test specimen into the plate/strip to respective wells.
6. For setting up positive and negative control reactions, Viral RNA (template) is replaced by HEV Positive Control and nuclease free water respectively. Refer the following table.

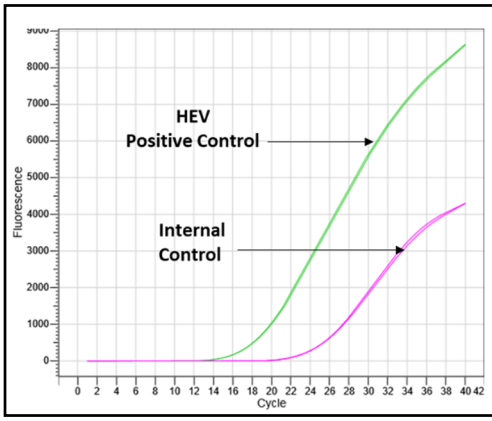
Set up of controls for the PCR run			
Components	**Product code**	**Volume for "1X" (One Reaction)**	
		Positive Control	**Negative Control**
Total PCR Reaction Mix	-	12.0 µL	12.0 µL
HEV Positive Control	DS1827	8.0 µL	-
Molecular Biology Grade Water	ML065	-	8.0 µL
Total reaction volume	**-**	**20.0 µL**	**20.0 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).

A. Recommended PCR program

1.	Reverse Transcription	:	50°C for 15 minutes	
2.	Initial denaturation	:	95°C for 2:30 minutes	
3.	Denaturation	:	95°C for 30 seconds	No. of cycles: 40
4.	Annealing	:	57°C for 30 seconds (Plate Read)	
5.	Plate Read	:	FAM/JOE	
6.	Hold	:	4°C for ∞	

B. Amplification Data



Sr. No.	Sample	Ct Values	
		Hepatitis E Virus (HEV)	Internal Control (IC)
1.	Positive Control (PC)	18.03	25.39
2.	Negative Control (NC)	-	25.73

The image shows an amplification plot for the Hepatitis E Virus (HEV) and internal control (IC) gene with associated Ct values generated by using Hi-PCR® Hepatitis E Virus (HEV) Probe PCR Kit on InstaQ 96 series instrument. The Ct values provided in the table are illustrative and may vary depending on the sample types.

C. Data Analysis

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

Control	Detection channel	
	FAM (HEV)	JOE (IC)
Positive Control	+	+
Negative Control	-	+

D. Data Interpretation

Interpret the results of the specimen as follows:

Detection Channel		Result Interpretation
FAM (HEV)	JOE (Internal Control)	
+ (Ct cut off ≤ 37)	+/-*	HEV Specific RNA detected
-	+	HEV specific RNA is not detected. Sample does not contain detectable amounts of HEV specific RNA.
-	-	PCR Inhibition or reagent failure. Retest the sample.

*Detection of the internal control (IC) in the JOE channel is not necessary for confirming positive results in the FAM channel. Presence of high HEV RNA load and/or PCR inhibitors in the original sample can result in a diminished or absent IC signal.

Performance Characteristics

Analytical sensitivity

Limit of detection (LOD)

The analytical sensitivity or the Limit of Detection (LOD) of Hi-PCR® Hepatitis E Virus (HEV) probe PCR Kit is defined as the concentration of HEV RNA molecules that can be detected with a positivity rate of ≥ 95%. The analytical sensitivity for Hi-PCR® Hepatitis E Virus (HEV) probe PCR Kit was conducted using synthetic HEV DNA and ATCC Quantitative Synthetic RNA from Hepatitis E virus (VR-3258SD). The preliminary LoD 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 detectable LoD. The data revealed that the Hi-PCR® Hepatitis E Virus (HEV) Probe PCR Kit detects ≈1 copy/µL. Thus, the detectable Limit of Detection (LoD) was determined to be ≈1 copy/µL. The sensitivity analysis of the Hi-PCR® Hepatitis E Virus (HEV) probe PCR Kit was carried out on HiMedia's InstaQ 96 series, Biorad's CFX Series and Thermo Fisher's QuantStudio™ 5 Real-Time PCR System.

Analytical Specificity

Inclusivity – In silico

The analytical specificity of the Hi-PCR® Hepatitis E Virus (HEV) Probe PCR Kit was validated through in silico analysis of the HEV primers and probes using NCBI BLAST, along with optimization of stringent PCR conditions. The primers and probes were examined for potential homologies with all sequences available in the NCBI database using multiple sequence alignment tools, ensuring that the kit detects all relevant HEV strains.

Analytical Reactivity

The analytical reactivity of the Hi-PCR® Hepatitis E Virus (HEV) Probe PCR Kit was verified through wet lab testing of the oligonucleotides (primers and probes) against commercially available HEV control-ATCC Quantitative Synthetic RNA from Hepatitis E virus (VR-3258SD).

Cross-Reactivity

Wet testing was conducted on genomic or synthetic DNA/RNA of various pathogens (from ATCC) using the InstaQ 96® Plus Real-Time PCR System to assess potential cross-reactivity. None of the pathogens listed in the table exhibited any reactivity with the primers and probes of the Hi-PCR® Hepatitis E Virus (HEV) Probe PCR Kit.

Human immunodeficiency virus 1 (HIV-1) (VR- 3245SD)	Human parainfluenza virus 1 strain C35 (VR-94DQ)
Hepatitis A virus (VR-3257SD)	Astrovirus (VR-3238SD)
Hepatitis B virus (VR-3232SD)	*Candida albicans* strain SC5314 (MYA-2876DQ)
Hepatitis C virus (VR-3233SD)	*Escherichia coli* (10798DQ)
Dengue virus (1-4) (VR-3228SD, VR-3229SD, VR-3230SD, VR-3231SD)	*Streptococcus pneumoniae* strain (ATCC 49619)
Rotavirus (VR-2018DQ)	*Cryptosporidium parvum* (PRA-67DQ)
Human papillomavirus 16 (VR-3240SD)	*Plasmodium falciparum* strain 3D7 (PRA-405)
Human papillomavirus 18 (VR-3241SD)	*Leptospira* (BAA-1198D-5)
SARS-CoV (VR-3276SD)	*Legionella pneumophila* subsp. (33152DQ)
Human adenovirus 1 strain Adenoid 71 (VR-1DQ)	Rhinovirus (VR-283DQ)
Norovirus (VR-3234SD)	*Giardia intestinalis* (30888D)
Enterovirus (VR-1826DQ)	Genomic DNA extracted from *Vibrio cholerae*
JC Polyomavirus (VR-1583DQ)	

Extraction kit compatibility:

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

HiMedia Viral Purification Nucleic Acid kits MB622MPF16, MB622MPF32, QIAamp Viral RNA Kits. Alternative nucleic acid extraction systems and kits might also be appropriate. The suitability of the nucleic acid extraction procedure for use with Hi-PCR® Hepatitis E Virus (HEV) Probe PCR Kit must 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 samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred in safety data sheets of the product. 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. This assay must not be performed on the specimen directly. Viral RNA should be extracted from sample using appropriate nucleic acid extraction method. Presence of PCR inhibitors and other interferences may lead to false negative or invalid results.

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 Hi-PCR® Hepatitis E Virus Probe PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Each lot of HiMedia's Hi-PCR® Hepatitis E Virus Probe PCR Kit is functionally tested in DNA amplification assays.

Troubleshooting Guide

Sr. No.	Problem	Cause	Solution
1.	No amplification	Degraded samples	Check the integrity of RNA using agarose gel electrophoresis.
			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 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

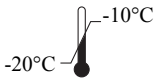
HiMedia's Hi-PCR® Hepatitis E Virus Probe PCR Kit is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

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

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