MBPCR148 Hi-PCR® Hepatitis A Virus (HAV) Probe PCR Kit

Instructions For Use

Description

Hepatitis A virus (HAV) is a non-enveloped single stranded RNA (ssRNA) virus. It causes acute infectious disease of the liver. Transmission via the fecal-oral route is the most common. Food samples can be contaminated via food handlers or by the environment. Due to their structural properties, HAV can be resistant to different physical/chemical treatments and food processes. Adsorbed on particles, they can survive in different kinds of environments for long periods of time. The consumption of contaminated food can lead to human outbreaks. There is no specific curative treatment but a vaccine is available. Serological methods have not been effectively adapted for HAV detection in environmental or food samples. Detection by cell culture is difficult. Detection by real time RT-PCR, after extraction and purification of the viral RNA, is a method of choice taking into account its speed and its sensitivity.

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

Intended Use

Recommended for sensitive and specific detection of Hepatitis A virus.

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® Hepatitis A Virus (HAV) Probe PCR Kit is designed to detect the **HAV RNA in FAM channel and Internal Control in JOE 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.

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.

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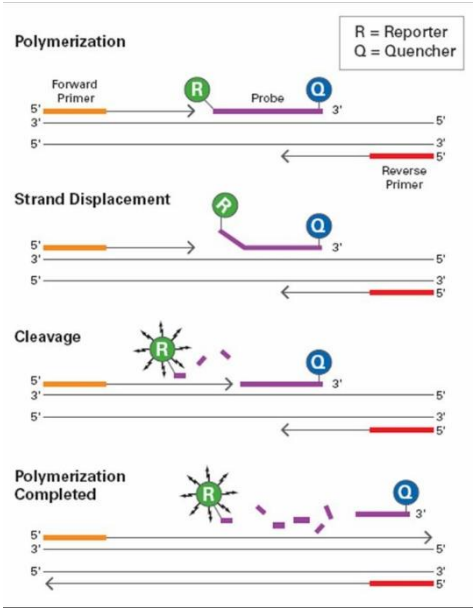
Diagrammatic representation of preferential binding of probe specific to DNA fragments in Real-time PCR



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
- Rapid detection of all relevant clinical pathogens

Types of Specimen: Water sample, Blood sample, Tissue sample, Food sample

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.

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, 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 DNA 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
RT Buffer	DS0221	108	216
10X solution H	DS0222	54	108
M-MuLV Reverse Transcriptase	DS0220	22	44
HAV Primer-Probe Mix	DS1244	81	162
HAV Positive Control	DS0653	40	80
Molecular Biology Grade Water for PCR	ML065	200	400

***** For a 20 µL PCR reaction

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
- For Processed Food Sample: HiPurA™ Food DNA Purification Kit (MB562)
- For Tissue Sample: HiPurA™ Mammalian Genomic DNA Purification Kit (MB506)
- For Blood Sample: HiPurA™ Mammalian Genomic DNA Purification Kit (MB504)
- For Virus culture: HiPurA™ Viral RNA Purification Kit (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	Volume to be added for 1R (for a 20 µL reaction)
RT Buffer	4
10X solution H	2.0
M-MuLV Reverse Transcriptase	0.8
HAV Primer-Probe Mix	3
Test Sample / Positive Control / Negative Control	8.0
Molecular Biology Grade Water for PCR	2.2
Total volume	20

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.	Reverse Transcription	:	50°C for 15 minutes	
2.	Initial denaturation	:	95°C for 2:30 minutes	
3.	Denaturation	:	95°C for 15 seconds	]
4.	Annealing	:	60°C for 30 seconds (Plate Read)	] No. of cycles: 40
5.	Plate Read	:	FAM/JOE	
6.	Hold	:	4°C for ∞	

Warning and 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 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® Hepatitis A Virus (HAV) Probe PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Each lot of HiMedia's Hi-PCR® Hepatitis A Virus (HAV) Probe PCR Kit is assayed for contaminating endonuclease, exonuclease and non-specific DNase activities. It has been functionally tested in DNA amplification assays.

Data Analysis

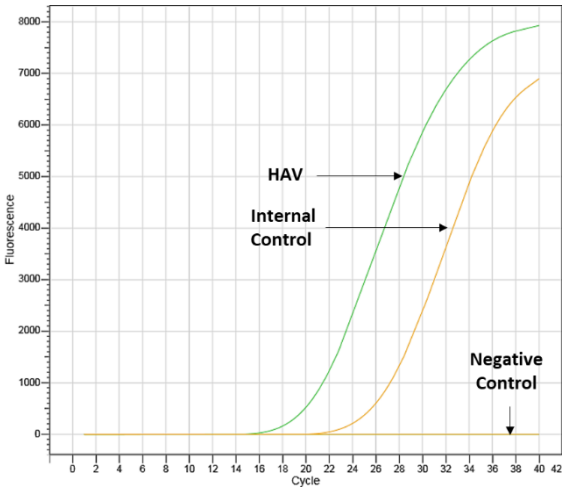
The following conditions should be met for a valid diagnostic test:

Control	Detection channel	
	FAM (Hepatitis A Virus)	**JOE (Internal Control)**
Positive Control	+	+/-*
Negative Control	-	+

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

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

Amplification Data



Sr. No.	Sample	C_t value
1.	HAV Positive control	19.86
2.	Internal Control	26.48

Image representing probe based real-time amplification data of HAV with C_t values (provided in table). The results completely depend upon sample types.

Performance Evaluation

Limit of Detection (LoD) - Analytical Sensitivity

The analytical sensitivity was defined as the lowest concentration of the target that could be reliably detected with 95% confidence. The analytical sensitivity for the Hi-PCR® Hepatitis A Virus (HAV) Probe PCR Kit was conducted using synthetic DNA on InstaQ96® Real Time PCR system. 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 detectable LoD. The data revealed that the Hi-PCR® Hepatitis A Virus (HAV) Probe PCR Kit detects ≈ 9 copies/µL. Thus, the detectable Limit of Detection (LoD) was determined to be 9 copies/µL or 9000 copies/ml.

Inclusivity - Analytical Sensitivity

In silico analysis for the assessment of inclusivity for the Hi-PCR® Hepatitis A Virus (HAV) Probe PCR Kit was conducted by mapping the primers and probes against all the available sequences in GenBank. The Hi-PCR® Hepatitis A Virus (HAV) Probe PCR Kit targets 100% of the known Hepatitis A Virus strains.

Cross-reactivity - Analytical Specificity

Wet testing analysis was performed against the pathogens available in the laboratory. In addition, *in silico* analysis was performed using NCBI nucleotide and Primer BLAST. The primers and probe for Hepatitis A Virus (HAV) were analyzed against the viruses related to HAV, organisms causing similar symptoms as an infection with HAV and organisms with similar route of transmission. Below mentioned table represents the list of pathogens analyzed for analytical specificity. No cross-reactivity was observed with any strains mentioned below.

Adenovirus	*Escherichia coli*	Norovirus G1
Campylobacter jejuni	Hepatitis B Virus	Norovirus G2
Candida albicans	Hepatitis C Virus	Rhinovirus
Chikungunya	Herpes simplex virus	Rotavirus
Cryptosporidium parvum	HPV16	*Salmonella typhi*
Dengue Serotype 1	HPV18	SARS-CoV
Dengue Serotype 2	Human immunodeficiency virus	*Shigella dysenteriae*
Dengue Serotype 3	*Klebsiella pneumoniae*	*Vibrio cholerae*
Dengue Serotype 4	*Legionella pneumophila*	
Enterovirus	*Leptospira*	

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 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 A Virus (HAV) 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.

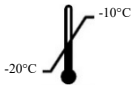
Disposal

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.

Symbols:

	Manufacturer		Do not use if package is damaged
	Batch code		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		Catalogue number

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