MBPCR151 Hi-PCR® Rotavirus Probe PCR Kit

Instructions For Use

Description

Rotavirus (RoV) is the most common cause of diarrheal disease which is contagious and is commonly caused in infants and young children. The virus belongs to the family Reoviridae. It is a double stranded RNA virus (dsRNA) with nine types, referred to as A, B, C, D, E, F, G, H and I. The virus is transmitted by the fecal-oral route. It causes gastroenteritis and infects and damages the cells that line the small intestine. Nucleic acid amplification-based assays or Polymerase Chain Reaction (PCR) is an alternative method that allows for sensitive and specific detection of RoV RNA from clinical and environmental 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 Hi-PCR® Rotavirus Probe PCR Kit is for *in-vitro* use only.

Intended Use

Recommended for sensitive and specific detection of Rotavirus 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 Hi-PCR® Rotavirus Probe PCR Kit is designed to detect the **RoV RNA in FAM channel** with **Internal Control in JOE channel** in a single tube reaction. The kit allows sensitive and specific detection of Rotavirus 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.

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

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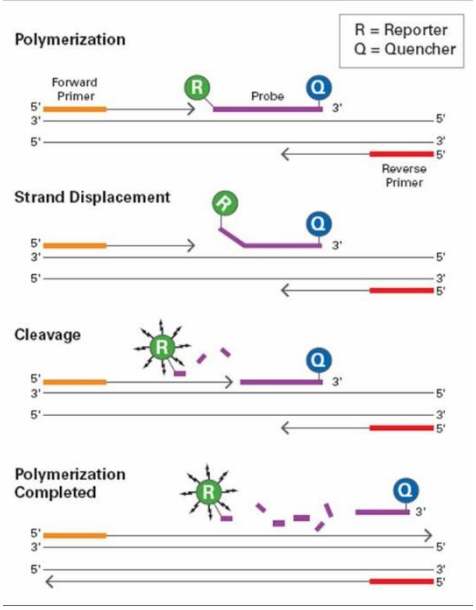
Diagrammatic representation of preferential binding of probe specific to DNA fragments in Real-time PCR



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

Sample Source: Water, Blood, Serum, Virus cultures, Tissue 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.

Sample Preparation

Various samples are routinely examined. For extraction and purification of pure RNA for high yield, perform the nucleic acid purification using HiMedia's extraction kits as instructed in the protocol.

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 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* (µL)	
		25R	50R
2X One Step Buffer Mix	DS1086	270	540
One-step RT Enzyme Mix	DS1087	22	44
Rotavirus Primer-Probe Mix	DS1272	33	65
Molecular Biology Grade Water for PCR	ML065	100	200
Rotavirus Positive Control	DS0768	40	80

***** 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 blood / serum / viral cultures / Tissue sample: HiPurA® Viral RNA Purification Kit (MB615)

Kit Compatibility with Real-Time PCR systems:

Hi-PCR® Rotavirus probe PCR Kit contains fluorophores compatible to:

- Insta Q96® - 6.0 Real time PCR System (HiMedia Laboratories Pvt. Ltd.)
- Insta Q96® Plus Real time PCR System (HiMedia Laboratories Pvt. Ltd.)
- CFX96™ Real-Time PCR Detection System (Bio-Rad Laboratories, Inc.)
- QuantStudio™ 3/5 Real-Time PCR Instrument (Applied Biosystems)

Note: Ensure that the Real-Time PCR system is calibrated for FAM dye, JOE dye and is maintained as 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 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	Product code	Volume to be added for 1R (for a 20 µL reaction)
2X One Step Buffer Mix	DS1086	10µL
One-step RT Enzyme Mix	DS1087	0.8 µL
Rotavirus Primer-Probe Mix	DS1272	1.2 µL
Template RNA / Positive Control / Negative Control	-	8.0
Total volume	**-**	**Upto 20 µL**

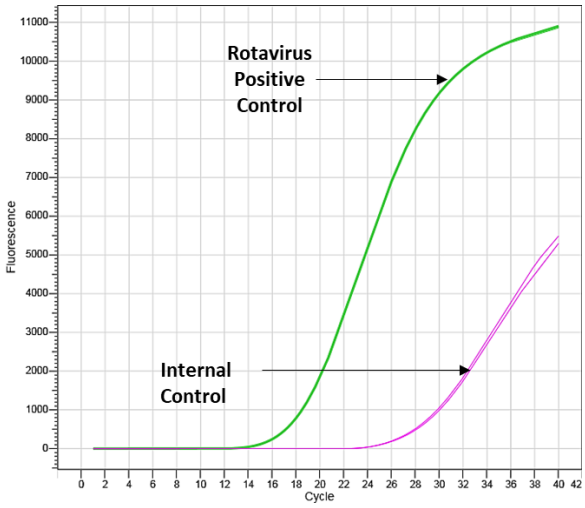
Centrifuge the tube briefly at 6000 rpm for about 10 seconds. Place the tubes in Real-time PCR machine and set the recommended PCR program (mentioned below). Interpret the data from the amplification plot (observe the Ct values).

B. Recommended PCR program

1.	Reverse Transcription	:	50°C for 15 minutes	
2.	Initial denaturation	:	95°C for 2 minutes 30 seconds	
3.	Denaturation	:	95°C for 15 seconds	No. of cycles: 45
4.	Annealing & Extension	:	60°C for 20 seconds (Sampling)	
	Channels	:	FAM/JOE[#]	
5.	Hold	:	4°C for ∞	

[#]For instruments not calibrated for JOE, HEX can be used.

C. Amplification Data



Sr No.	Sample	Ct Value
1.	Rotavirus Positive Control	17.75
2.	Internal Control	28.68

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

D. Data Analysis:

Detection Channel		Result Interpretation
FAM (Rotavirus)	JOE (Internal Control)	
+	+/-*	Positive for Rotavirus
-	+	Negative for Rotavirus
-	-	PCR inhibition or reagent failure. Repeat PCR or repeat extraction from original sample

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

*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.

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® Rotavirus (RoV) 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® Rotavirus (RoV) Probe PCR Kit detects ≈ 4 copies/µL. Thus, the detectable Limit of Detection (LoD) was determined to be 4 copies/µL.

Inclusivity - Analytical Sensitivity

In silico analysis for the assessment of inclusivity for the Hi-PCR® Rotavirus (RoV) Probe PCR Kit was conducted by mapping the primers and probes against all the available sequences in GenBank. The Hi-PCR® Rotavirus (RoV) Probe PCR Kit targets 100% of the known Rotavirus 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 Rotavirus (RoV) were analyzed against the viruses related to RoV, organisms causing similar symptoms as an infection with RoV 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.

Campylobacter jejuni	*Plasmodium falciparum*	Human papillomavirus 16
Candida albicans	*Salmonella typhi*	Human papillomavirus 18
Chikungunya	*Shigella flexineri*	Human immunodeficiency virus 1B
Cryptosporidium parvum	*Leptospira*	Human immunodeficiency virus 1A
Dengu Serotype 1	*Legionella pneumophila*	Parainfluenza
Dengu Serotype 2	Adenovirus	Astrovirus
Dengu Serotype 3	Hepatitis B	Norovirus G2
Dengu Serotype 4	Hepatitis C	Rhinovirus
Vibrio cholerae	Hepatitis E	Rotavirus
Escherichia coli	Hepatitis A	SARS-CoV
Streptococcus pneumoniae	Herpes simplex virus	Enterovirus

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.

Evaluation

Each lot of HiMedia's Hi-PCR® Rotavirus Probe PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Each lot of HiMedia's Hi-PCR® Rotavirus Probe PCR Kit is assayed for contaminating endonuclease, exonuclease and non-specific DNase activities. The kit has been functionally tested in DNA amplification assays.

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® Rotavirus 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.

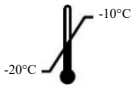
Disposal

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
	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		

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