MBPCR156 Hi-PCR® Vibrio cholerae Probe PCR Kit

Instructions For Use

Description

Vibrio cholerae is a Gram-negative, non-spore forming, flagellated, facultative anaerobe. It dwells in brackish or saltwater as it is highly halophilic requiring salt-rich environment for thriving. This bacterium infects the intestine and increases mucous production causing diarrhea and vomiting which result in extreme dehydration. *V. cholerae* enters the human body through ingestion of contaminated food or water. It causes cholera, an infectious disease plaguing many developing nations, areas of poor sanitation and can be endemic, epidemic or pandemic. Being an acute diarrhoeal disease, Cholera can kill within hours if left untreated. As per WHO report, every year approximately, 1.3 to 4.0 million cases of cholera, and 21 000 to 143 000 deaths occur worldwide due to cholera. Specific and faster methods for detection, such as real–time PCR, are the need of an hour. These techniques help to detect targeted pathogens quickly; and helps to take further actions.

NOTE: HiMedia's Hi-PCR® Vibrio cholerae Probe PCR Kit is for *in-vitro* use only.

Intended Use

Recommended for sensitive and specific detection of *V. cholerae* in clinical and environmental 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 having a fluorescent reporter dye attached to the 5' end and a quencher dye to the 3' end. HiMedia's Hi-PCR® Vibrio cholerae Probe PCR Kit is designed to detect the ***V. cholerae* specific gene in FAM channel** and **Internal Control in JOE channel** in a single tube reaction. The kit allows sensitive and specific detection of *V. cholerae* 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.

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 1 reaction of negative control per run.

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

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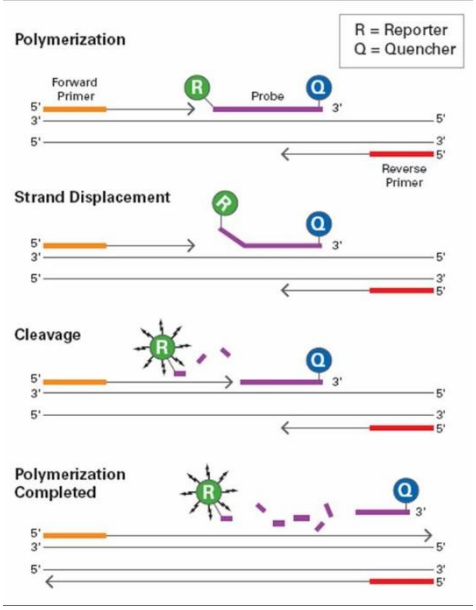
Internal Control

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 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: Bacterial Culture / Water / Blood/ Food samples

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
Vibrio cholerae Mastermix	DS1597	270	540
Vibrio Primer-Probe Mix	DS1425	54	108
Molecular Biology Grade Water for PCR	ML065	50	100
Vibrio Positive Control	DS1599	43	86

***** For a 20 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

Various samples are routinely examined. For extraction and purification of high yield and pure bacterial DNA, perform the nucleic acid purification using HiMedia's extraction kits as instructed 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) & Sealing film (PR18)
- Insta Q Real Time PCR System (Product Code: LA1012/LA1073/LA1023/LA1024/LA1074)
- Barrier Micropipette Tips (Product Code: LA749 / LA749A / LA751 / LA751A / LA750 / LA750A / LA859 / LA859A)
- Micropipettes
- For clinical samples: HiPurA® Multi-Sample DNA Purification Kit (MB554)
- For cultured isolates: HiPurA® Bacterial Genomic DNA Purification Kit (MB505)
- For Food samples: HiPurA® Food Pathogen (Bacteria) DNA Purification Kit (MB568A) and HiPurA® Food DNA Purification Kit (MB562)
- For Water samples: HiPurA® Water DNA Purification Kit (with enrichment) (MB547) and HiPurA® Water DNA Purification Kit (without enrichment) (MB577)

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 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.

A. Protocol for PCR Master Mix Preparation

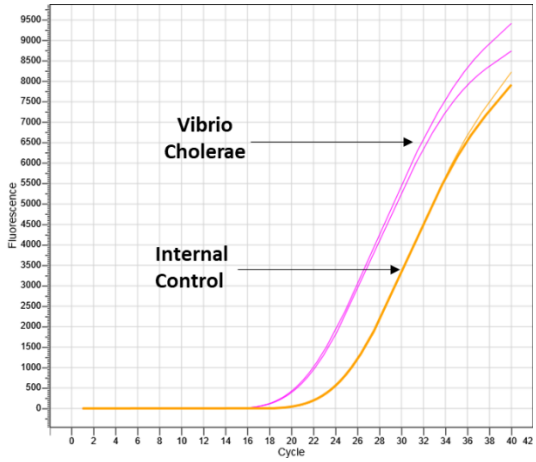
Components	Product Code	Volume to be added for 1R (for a 20 µL reaction)
Vibrio cholerae Mastermix	DS1597	10µL
Vibrio Primer-Probe Mix	DS1425	2 µL
Template DNA/Negative Control/Positive Control	-	8µL
Total volume	-	Upto 20 µL

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.	Initial denaturation	:	95°C for 10 minutes	
2.	Denaturation	:	95°C for 15 seconds	No. of cycles: 40
3.	Annealing and Extension	:	60°C for 30 seconds	
	Plate Read	:	FAM/JOE	
4.	Hold	:	4°C for ∞	

C. Amplification Data



Sr. No.	Sample	C_t value	
		PC	NC
1.	*V. cholerae*	21.1	-
2.	Internal Control	24.5	24.2

PC: Positive Control NC: Negative Control

Image representing amplification plot of *V. cholerae* DNA with Ct values using HiMedia's Hi-PCR® Vibrio cholerae Probe PCR Kit. The results completely depend upon sample types.

D. Data Analysis

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

Control	Target	
	***V. cholerae* (FAM)**	**Internal Control (JOE)**
Positive Control	+	+
Negative Control	-	+

Ct value	Result
≤ 36	Detected (+)
> 36 or N/A	Not detected (-)

E. Data Interpretation

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

*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.

Analytical Performance

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® *Vibrio cholerae* Probe PCR Kit was conducted using genomic 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® *Vibrio cholerae* Probe PCR Kit detects ≈ 1 copy/µL. Thus, the detectable Limit of Detection (LoD) was determined to be 1 copy/µL.

Inclusivity

In silico analysis for the assessment of inclusivity for the Hi-PCR® Vibrio cholerae Probe PCR Kit was conducted by mapping the primers and probe against the available *Vibrio cholerae* sequences in GenBank. The Hi-PCR® Vibrio cholerae Probe PCR Kit targets 100% of the known *Vibrio cholerae* 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 *Vibrio cholerae* were analyzed against the organisms related to *Vibrio cholerae*, organisms causing similar symptoms as an infection with *Vibrio cholerae* 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	Pseudomonas aeruginosa
Candida albicans	Acinetobacter baumannii
Escherichia coli O157:H7	Staphylococcus epidermidis
Wild Escherichia coli	Listeria monocytogenes
Streptococcus pneumoniae	Lactobacillus acidophilus
Salmonella typhi	Bacillus subtilis
Shigella flexineri	Aspergillus fumigatus
Legionella pneumophila	Saccharomyces cerevisiae
Staphylococcus aureus	Enterococcus faecalis
Klebsiella pneumoniae	

Warning

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

Precautions

Read the procedure carefully before starting the experiment. 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.

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® Vibrio cholerae Probe PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Quality Control
Each lot of HiMedia's Hi-PCR® Vibrio cholerae Probe PCR Kit is assayed for contaminating endonuclease, exonuclease and non-specific DNase activities. Functionally tested in RNA amplification.

Troubleshooting Guide:

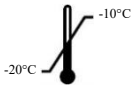
Sr. No.	Problem	Cause	Solution
1.	No amplification	Degraded samples	1. Check the integrity of DNA using agarose gel electrophoresis.
			2. Use freshly prepared DNA to ensure the availability of intact template sequence for efficient amplification.
		Error in protocol setup	Verify that the correct reagent 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® Vibrio cholerae 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		

Authorized representative (AR) Address :

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