MBPCR027 Vibrio cholerae Detection Kit (Real-Time)

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 bacteria 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 which can be endemic, epidemic or pandemic.

V. cholerae has an accessory protein, Neuraminidase, which enhances the effects of the cholera enterotoxin (CT), a toxin responsible for the diarrheal disease. Presence of virulence genes in environmental strains of *V. cholerae* viz. **CTXAB gene** is known to play a cardinal role in maintaining virulence in *Vibrio cholerae*. This gene is believed to be exclusively associated with clinical strains of different serogroups.

Specific and faster methods for detection foodborne pathogens, such as real–time PCR, are the need of an hour. These techniques help to detect targeted pathogens quickly; this early and precise detection helps to take further actions.

NOTE: The Vibrio cholerae Detection Kit (Real-Time) is for *in vitro* use only.

Principle :

The Vibrio cholerae Detection Kit (Real-time) is designed for detection of specific sequence of **CTXAB (779 bp)** gene of *Vibrio cholerae*. Cholera enterotoxin (CT) has A and B subunit toxins: toxin A is responsible for enzymatic and intracellular functions, while toxin B is responsible for binding the toxin to the eukaryotic cell receptor.

This **CTXAB gene** is responsible for identification of *Vibrio cholerae* giving an amplification of **779bp** product. Vibrio cholerae Detection Kit (Real-time) testing provides rapid, sensitive and specific detection of *V. cholerae.*

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 Polymerase Chain Reaction. This technique is used to amplify and simultaneously quantitate a targeted DNA sequence. Real-time PCR systems based on SYBr Green assays have increasingly been used for accurate, reliable detection and quantitation of various food-borne pathogens. HiMedia's Vibrio cholerae Detection Kit (Real-time), is one such SYBr green based qPCR techinique which allows amplification of **CTXAB gene.**

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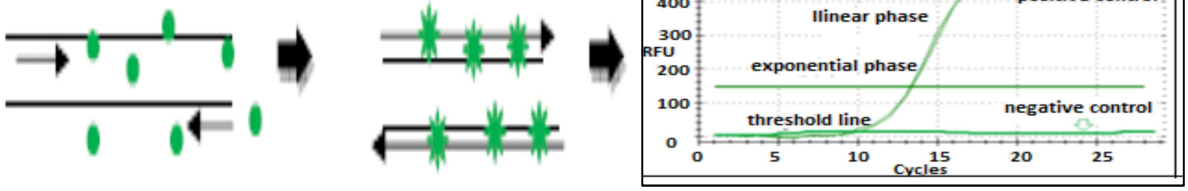
A. Diagrammatic representation of preferential binding of SYBr Green Dye to specific DNA fragments in real-time PCR.

a) Dye in solution emits low fluorescence

b) Emission of the fluorescence by binding

c) Amplification data



SYBr Green dye cycles between an unbound (Denaturation step) and a bound (Annealing through Extension) state as the reaction progresses. Signal intensity increases as the quantity of amplicons increase in later cycles indicating amplification. During elongation, more and more dye molecules bind to the newly synthesized DNA. If the reaction is monitored continuously, an increase in fluorescence is viewed in real-time. Upon denaturation of the DNA for the next heating cycle, the dye molecules are released and the fluorescence signal falls.

Keys : SYBr
Forward primer
Reverse primer
DNA Strand

Features:

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

Kit Contents:

The provided PCR kit contains:

Components	**Reagents provided for 10R (reactions)***	**Reagents provided for 25R (reactions)***
Hi-SYBr master mix (2X master mix containing SYBr Green, Assay buffer, Taq Polymerase, $MgCl_2$, dNTPs) **(MBT074)**	150 µl	400 µl
Primer Mix	25 µl	60 µl
Nuclease free water **(ML065)**	1 ml	2 ml

***** For a 20µl PCR reaction

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.

Sampling and Handling:

Sample Preparation:

Various food, clinical and environmental samples as well as cultured bacteria are routinely examined.

For extraction and purification of high yield and pure bacterial DNA, perform the nucleic acid purification using HiMedia's **HiPurA™ Bacterial Genomic DNA Purification Kit (MB505)** as instructed in the protocol.

Flow Chart for setting up PCR Reaction

Add 10µl Hi-SYBr master mix **(MBT074)** in a PCR tube

⇩

Add 2 µl the Primer mix (Final concentration 10 pmoles provided)

⇩

Add 1-2 µl template DNA (upto 50 ng of extracted DNA)

⇩

Add nuclease free water (ML065) to make the final volume to 20 µl

⇩

Centrifuge the tube briefly at 6000 rpm for about 10 seconds.

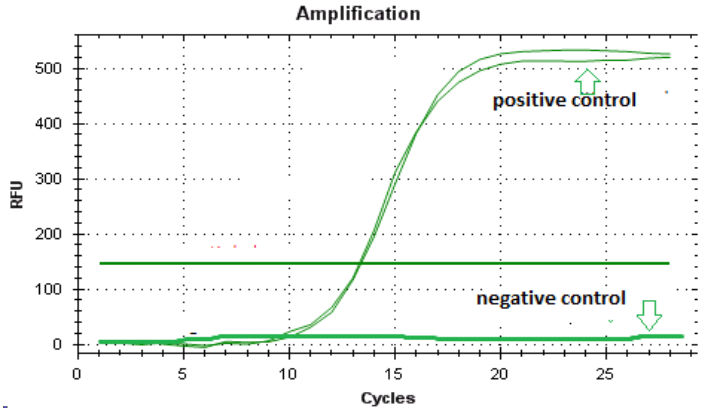
⇩

Place the tubes in real-time PCR machine and set the recommended PCR program

⇩

Interpret the data from the amplification plot (observe the Ct values)

Amplification Data:



Sr. No.	Sample	C_t value
1	Negative control	N/A
2	1 µl of template DNA (amplicon of *V. cholerae*)	13.36
3	1 µl of template (amplicon of *V. cholerae*)	13.29

Figure: Data representing real-time amplification data of *Vibrio cholerae* with C_t values (provided in table).

C. Recommended PCR program:

1. Initial denaturation : 95°C for 10 minutes

2. Cycling Parameters (No. of cycles: 30)
   Denaturation : 95°C for 30 seconds
   Annealing : 56°C for 30 seconds
   Extension : 72°C for 45 seconds

3. Final Extension : 72°C for 10 minutes.
4.

Sensitivity: Detectable upto 100-1000 CFU / ml (mg).

Storage:

The provided kit has a shelf-life of 6 months when stored at -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.

Quality Control:

Each lot of HiMedia's Vibrio cholerae Detection Kit (Real-time) is assayed for contaminating endonuclease, exonuclease and non-specific DNase activities. Functionally tested in DNA 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 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.

Safety Information

The Vibrio cholerae Detection Kit (Real-time) is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

Product Use Limitation & Warranty

HiMedia guarantees the performance of Vibrio cholerae Detection Kit (Real-time) in the manner described in the product literature. The kit is designed, sold for research and for *in vitro* purposes only. No claim or representation is intended to provide information for the diagnosis, prevention or treatment of a disease.
All due care and attention should be exercised in the handling of the products. We recommend all users of HiMedia products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

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.

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