# MBPCR264 Hi-PCR® Vibrio parahaemolyticus Semi-Q PCR Kit

**Description**

*Vibrio parahaemolyticus* is a Gram-negative halophilic bacterium belonging to the genus *Vibrio*. It is ordinarily found in estuarine, marine and coastal environments, but can also be found in livestock and poultry meat, freshwater fish, preserved eggs, and pickles as a contaminant. It is a major food-borne pathogen causing stomachache, diarrhea, vomiting, dehydration, chills, and fever after the consumption of contaminated raw or undercooked seafoods including crab, shrimp, shellfish, lobster, fish, and oysters. Recently, *V. parahaemolyticus* was reported as a major causative agent of acute hepatopancreatic necrosis syndrome troubling penaeid shrimp, thereby inflicting major loses to the shrimp aquaculture industry. An early detection of *V. parahaemolyticus* contamination is therefore a prerequisite. To meet enhanced diagnostic accuracy within a shorter time as compared to traditional methods, Semi-Q PCR method is developed for detection of *V. parahaemolyticus* in food samples.

**NOTE:** The Hi-PCR® *Vibrio parahaemolyticus* Semi-Q PCR Kit is for research use only.

**Intended Use**

Recommended for sensitive and specific detection of *Vibrio parahaemolyticus* in food samples.

**Principle**

Polymerase chain reaction is used to amplify a targeted DNA sequence by use of specific primers. HiMedia's Hi-PCR® *Vibrio parahaemolyticus* Semi-Q PCR Kit is a qualitative conventional PCR kit which allows amplification of a *V. parahaemolyticus* specific target gene along with the internal control in a single tube reaction. The amplified target of 341 bp product size is confirmed by using agarose gel electrophoresis. This kit also contains an **Internal control** and a **Positive control**.

**Positive control**

This is a control reaction using a known template. 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 pathogen) 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
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**Features**

- Fast and simple
- Good sensitivity and specific results
- Guaranteed reproducible results

**Sample Source:** Food sample

**Storage and Shelf life**

The provided kit has a shelf-life of 12 months when stored between -15°C to -25°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 contains reagents for 25 µL/reaction volume:

| Components | Product Code | Reagents provided for (reactions)* | |
|---|---|---|---|
| | | 25R | 50R |
| 2X PCR TaqMixture | MBT061 | 338 | 675 |
| V. parahaemolyticus primer mix | DS1661 | 68 | 135 |
| V. parahaemolyticus Positive Control | DS1663 | 25 | 50 |
| Water | DS0440 | 135 | 293 |
| 6X Gel Loading Buffer | ML015 | 54 | 108 |
| 100 bp DNA Ladder | MBT049 | 20 | 40 |

**Specimen collection and Handling**

Follow appropriate techniques for handling contaminated food specimens; after use, contaminated materials must be sterilized by autoclaving before discarding. Standard precautions as per established guidelines should be followed while handling contaminated food specimens. Safety guidelines may be referred to in individual safety data sheets.

**Sample Preparation**

For preparation of bacterial DNA, perform nucleic acid purification using HiPurA® Multi-Sample DNA Purification Kit (MB554) as described 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)
- Thermal Cycler (Product Code: LA948/ LA949/ LA950/ LA1006/ LA1015/ LA1059/ LA1060/ LA1066)
- Barrier Micropipette Tips (Product Code: LA749/ LA749A/ LA751/ LA751A/ LA750/ LA750A/ LA859/ LA859A)
- Micropipettes

**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.
- Centrifuge the components briefly once thawed.

**A. Protocol for PCR Master Mix Preparation**

Perform PCR reactions for each DNA sample as per the following table:

| Components | Product Code | Recommended volume to be added per reaction (µL) |
|---|---|---|
| 2X PCR TaqMixture | MBT061 | 12.5 |
| V. parahaemolyticus primer mix | DS1661 | 2.5 |
| Template DNA / Positive Control / Negative | - | 5 |
| Water | DS0440 | 5 |
| Total volume | | 25 |

NOTE: (Optional) – The user can also set up an additional PCR reaction containing 1µL of Positive control DNA (provided) in a separate tube.

Centrifuge the tube briefly at 6000 rpm for about 10 seconds and place the tubes in the PCR machine and set the recommended PCR program (mentioned below). Interpret the data using Agarose Gel Electrophoresis.

**B. Recommended PCR program**

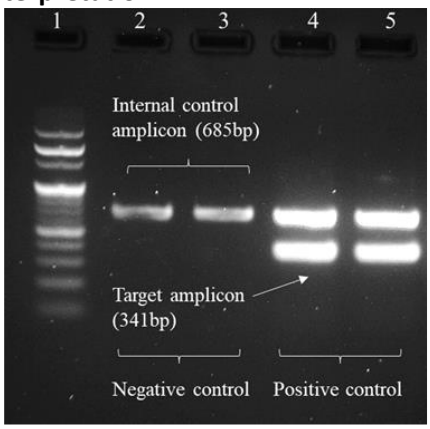
| | | | | |
|---|---|---|---|---|
| 1. | Initial denaturation | : | 95°C for 5 minutes | |
| 2. | Denaturation | : | 95°C for 30 seconds | No. of cycles: 35 |
| 3. | Annealing | : | 63°C for 20 seconds | |
| 4. | Extension | : | 72°C for 30 seconds | |
| 5. | Final extension | : | 72°C for 10 minutes | |

After amplification, the products can be kept at 4°C overnight or frozen at -20°C for long-term storage.

**C. Visualizing PCR Assay Results**

- For analysis of the PCR results, load 5 µL of amplicon on a 1.5% agarose gel along with 1 µL of 6X Gel loading buffer (ML015). Load 3 µL of 50 bp DNA ladder (MBT084) in a separate well.
- Incorporate EtBr in the agarose gel or stain the agarose gel after electrophoresis using EtBr solution for 10-15 mins. Confirm the expected amplicon size by comparing with 50 bp DNA marker.

**Data Interpretation:**




| LANES | CONTENT |
|---|---|
| 1 | 100 bp Ladder |
| 2-3 | Negative control |
| 4-5 | Positive control |

**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 contaminated food samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred in safety data sheets of the product.

**Performance and Evaluation**

Each lot of HiMedia's Hi-PCR® *Vibrio parahaemolyticus* Semi-Q PCR Kit is tested against predetermined specifications to ensure consistent product quality.

**Quality Control**

Each lot of HiMedia's Hi-PCR® *Vibrio parahaemolyticus* Semi-Q PCR Kit is assayed for contaminating endonuclease, exonuclease and non-specific DNase activities. It 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 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 | 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.<br>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 parahaemolyticus* Semi-Q PCR Kit is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

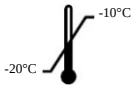
**Disposal**

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 contaminated food 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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