

## MBPCR002

## Hi-PCR® E. coli O157:H7 Semi-Q PCR Kit

### Description:

*E. coli O157:H7* is a rod-shaped, gram-negative, aerobic and non-fermentative bacterium that causes hemolytic colitis and hemolytic uremic syndrome. *E. coli O157:H7* is known by its somatic (cell wall) antigen (O157) and its flagella antigen (H7). *E. coli O157:H7* is also known to produce Shiga-like toxins which cause severe symptoms. The main mode of infection is through food, untreated water and unpasteurized milk. This organism is characterized by Endotoxin shock as one of the lethal manifestation of infection. While majority of the patients recover from infection around 15% of the patients develop hemolytic uremic syndrome which could be fatal.

**NOTE: The Hi-PCR® E. coli O157:H7 Semi-Q PCR Kit is for *in vitro* use only.**

### Intended Use:

The Hi-PCR® E. coli O157:H7 Semi-Q PCR Kit is designed for detection of specific sequence for *E. coli O157:H7* in various food sources, cells, environmental and clinical samples. Conventional PCR testing can provide rapid, sensitive and specific detection of *E. coli O157:H7*.

### Principle:

HiMedia's Hi-PCR® E. coli O157:H7 Semi-Q PCR Kit is a qualitative conventional PCR kit which allows the amplification **eaеA** gene (**450 bp**) using specific primers. The amplified target is detected by using agarose gel electrophoresis. This kit also contains **Internal control** and **Positive control**.

**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.

**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.

Polymerase Chain Reaction (PCR) is a very sensitive and specific method for amplification based detection of genes. The three steps of a successful PCR reaction include Denaturation, Annealing and Extension. The double-stranded DNA melts and forms single stranded DNA at a high temperature (Denaturation). Sequence-specific primers bind to the target sequence on single-stranded DNA at lower temperature (Annealing). Taq DNA Polymerase adds dNTPs onto the single stranded DNA at intermediate temperature (Extension). These 3 steps of PCR are usually repeated between 25 to 40 times in each PCR assay.

Gel electrophoresis is used to analyze the amplification of desired gene region for target pathogen based on separation of DNA fragments according to their size.

**Features:**

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

**Sample Source:** food sources, cells, environmental and clinical samples

**Storage:**

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 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.

**Kit Contents:**

The provided PCR contains:

Components	Product code	Reagents provided for (reactions)* (μL)	
		25R	50R
Hi-Quanti 2X Realtime PCR Mastermix	MBT180	675	1350
Primer Mix for <i>E. coli</i> O157:H7	DS0126	54	108
Primer Mix for Internal Control (685 bp)	DS0354	54	108
Molecular Biology Grade Water for PCR	ML065	490	980
6X Gel Loading Buffer	ML015	54	108
100 bp DNA Ladder	MBT049	20	40
Positive control ( <i>E. coli</i> O157:H7 DNA )	DS0353	5	10
Internal Control DNA	DS0123	27	54

\* For a 50μl 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 food source samples, environmental samples, clinical materials, cultured bacteria and human fecal specimens can be examined. For preparation of bacterial DNA perform the nucleic acid purification using HiMedia's HiPurA<sup>®</sup> Bacterial DNA Purification Kit (MB505) 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)
- 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, suitable amount of all PCR components should be completely thawed on ice (4°C).
- Perform the amplification reactions in a clean area.
- Use of aerosol barrier pipette tips is recommended to reduce contamination risks from extraneous DNA templates.
- Extract and store positive control material (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 reaction for each DNA sample as per the following table:

Components	Recommended volume to be added per reaction (µL)
Hi-Quanti 2X Realtime PCR Mastermix ( <b>MBT180</b> )	25 µL
Primer Mix for <i>E. coli</i> O157:H7 ( <b>DS0126</b> )	2 µL
Primer Mix for Internal Control (685 bp) ( <b>DS0354</b> )	2 µL
Template DNA	2 µL
Internal Control DNA ( <b>DS0123</b> )	1 µL
Molecular Biology Grade Water for PCR ( <b>ML065</b> )	Up to 50 µL

**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 10 minutes | No. of cycles: 1    |
| 2 Denaturation         | : 94°C for 45 seconds | } No. of cycles: 30 |
| 3 Annealing            | : 56°C for 30 seconds |                     |
| 4 Extension            | : 72°C for 30 seconds |                     |
| 5 Final Extension      | : 72°C for 10 minutes | No. of cycles: 1    |

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

#### D. *E. coli* O157:H7 PCR Assay Results Interpretation

- For analysis of the PCR data, load 10 µl of amplicon on a 1.5% Agarose gel along with 1 µl of 6X Gel Loading Buffer (ML015).
- Load 4 µl of 100 bp DNA ladder (MBT049) in separate well.

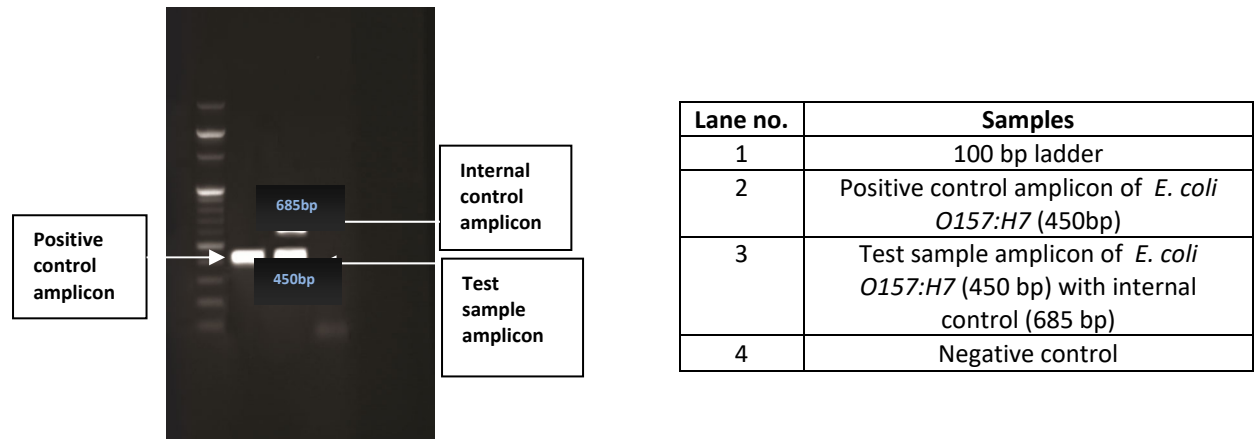
#### E. EtBr-staining to check results

- Incorporate EtBr in the agarose gel or stain the agarose gel with EtBr for 10-15 min.
- Confirm the expected amplicon size comparing with 100 bp DNA marker.

## F. Quality Control:

Each lot of HiMedia's Hi-PCR® *E. coli* O157:H7 Semi-Q PCR Kit is assayed for contaminating endonuclease, exonuclease and non-specific DNase activities. Functionally tested in DNA amplification.

## G. Amplification Data:



Gel image representing amplification of *eae* gene using target sample of *E. coli* O157:H7 with positive control (450bp) and internal control (685bp)

## 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.

## Performance and Evaluation

Each lot of HiMedia's Hi-PCR® *E. coli* O157:H7 Semi-Q PCR Kit is tested against predetermined specifications to ensure consistent product quality.

### 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.
		Error in reaction set-up	Prepare large volume reaction mix, vortex thoroughly and aliquot appropriately into reaction tubes.
2.	Variability between replicates	Air bubbles in reaction mix	Briefly centrifuge reaction samples/plate prior to running on a PCR machine.
		Pipetting error	Replicates can show increased variation due to poor laboratory techniques or imprecise pipettes.
		Reagents contaminated	1. Replace all critical solutions. 2. Repeat the analysis of all tests with fresh aliquots of critical reagents.
3.	Amplification in negative control		

### Safety Information

The Hi-PCR® E. coli O157:H7 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 clinical sample must be decontaminated and disposed off 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](mailto: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 :

	<b>AR Experts B.V.</b> Boeingavenue 209, 1119 PD, Schiphol-Rijk, The Netherlands
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### Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory, diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.

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