E. coli O157: H7 Detection 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 *E. coli* O157:H7 Detection Kit is for *in vitro* use only.

**Intended Use:**

The *E. coli* O157:H7 Detection Kit is designed for detection of specific sequence of *eaeA* gene (450 bp) 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. 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.

This diagnostic kit assures sensitive detection in clinical samples. The kit is designed for *in vitro* diagnostics and provides qualitative detection.

**Principle:**

HiMedia's *E. coli* O157:H7 Detection Kit is a qualitative conventional PCR kit which allows the amplification *eaeA* gene (450 bp) using specific primers. The amplified target is detected by using agarose gel electrophoresis.

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

Unit Definition:

1 u is defined as amount of enzyme that is required to catalyze the incorporation of 10 nmoles of dNTP into acid-insoluble material in 30 minutes at 74°C.

Storage and Shelf-life:

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. The kit provided is stable for 6 months when stored at mentioned conditions. HiMedia does not recommend using the kit after the expiry date stated on pack.

Kit Contents:

The provided PCR contains:

<table>
<thead>
<tr>
<th>Components</th>
<th>Reagents provided for 10R (reactions)</th>
<th>Reagents provided for 25R (reactions)</th>
<th>Reagents provided for 50R (reactions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2X PCR Master Mix (MBT061)</td>
<td>260 µl</td>
<td>650 µl</td>
<td>1.5ml</td>
</tr>
<tr>
<td>Primer Mix for <em>E. coli</em> O157:H7</td>
<td>25 µl</td>
<td>60 µl</td>
<td>120 µl</td>
</tr>
<tr>
<td>Primer Mix for Internal Control</td>
<td>25 µl</td>
<td>60 µl</td>
<td>120 µl</td>
</tr>
<tr>
<td>Nuclease free water (ML065)</td>
<td>1 ml</td>
<td>2 ml</td>
<td>4ml</td>
</tr>
<tr>
<td>6X Loading Dye (ML015)</td>
<td>100 µl</td>
<td>200 µl</td>
<td>400 µl</td>
</tr>
<tr>
<td>100 bp DNA Ladder (MBT049)</td>
<td>40 µl</td>
<td>90 µl</td>
<td>180 µl</td>
</tr>
<tr>
<td>Positive control (E. coli O157:H7 DNA)</td>
<td>15 µl</td>
<td>35 µl</td>
<td>65 µl</td>
</tr>
<tr>
<td>Internal Control DNA</td>
<td>15 µl</td>
<td>35 µl</td>
<td>65 µl</td>
</tr>
</tbody>
</table>

Sample Collection and 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™ Bacterial DNA Purification Kit (MB505) as described in the protocol.

Enrichment of pathogens (if required):

- In order to ensure sensitive detection of pathogens from different variety of food products by PCR, the pathogens need to be enriched in broth.
For any raw or cooked solid food samples, follow the procedure below:

- Weigh 25g of material and add it to 225 ml of the broth and for liquid samples like milk, aliquot 25 ml in 225ml of the broth.
- Incubate at 37°C for 16-18 hours with shaking.
- Transfer 1 ml of enriched sample in 1.5 ml tube.
- Centrifuge at 12,000 rpm for 5 minutes and discard the supernatant.
- Add 200 µl of sterile D/W. Mix well with vortexing. Centrifuge at 12,000 rpm for 5 minutes and discard the supernatant.
- Add 150 µl of sterile D/W or TE buffer (10 mM Tris and 0.5 mM EDTA, pH 7.6) and mix well by vortexing.
- Boil at 95°C for 5 minutes.
- Centrifuge at 12,000 rpm for 5 minutes and take the supernatant.
- Use 2-5 µl of the supernatant as PCR template.

For any Clinical samples or isolates, follow the procedure below:

- Inoculate a single colony or loopful of culture in selective enrichment media for E. coli O157:H7.
- Incubate overnight for around 12-16 hours at 37°C with shaking.
- Harvest 1 to 1.5 ml of overnight grown culture and perform the nucleic acid purification using HiMedia’s HiPurA Bacterial DNA Extraction kit (MB505) as described in the protocol.

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:

**Preparation of PCR Reaction Mixture**

Add 25 µl of 2X PCR Master Mix (MBT061) in a PCR tube

In the same tube, add 2 µl of E. coli O157:H7 primer mix + 2 µl internal control primer mix (10 pmoles concentration provided)

Add 1-2 µl of template DNA (upto 50 ng of extracted DNA) and add 1µl of Internal Control DNA (provided)

Add nuclease free water (ML065) to make the final volume to 50 µl
Centrifuge the tube briefly at 6000 rpm for about 10 seconds.

Place the tubes in the PCR machine and set the recommended PCR program. (mentioned below)

Interpret the data using Agarose gel electrophoresis

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

B. Recommended PCR program:

1. Initial denaturation : 94°C for 10 minutes

2. Cycling Parameters (No. of cycles: 27)
   - Denaturation : 94°C for 45 seconds
   - Annealing : 56°C for 30 seconds
   - Extension : 72°C for 30 seconds

3. Final Extension : 72°C for 10 minutes

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 DNA loading dye (ML015).
- Load 3 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

<table>
<thead>
<tr>
<th>Lane no.</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100 bp ladder</td>
</tr>
<tr>
<td>2</td>
<td>Positive control amplicon of <em>E. coli</em> O157:H7 (450bp)</td>
</tr>
<tr>
<td>3</td>
<td>Test sample amplicon of <em>E. coli</em> O157:H7 (450 bp) with internal control (685 bp)</td>
</tr>
<tr>
<td>4</td>
<td>Negative control</td>
</tr>
</tbody>
</table>

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

Sensitivity: Detectable upto 10-1000 cfu/ml (mg) before pre-enrichment.

Quality Control:

Each lot of HiMedia E. coli O157:H7 Detection Kit is assayed for contaminating endonuclease, exonuclease and non-specific DNase activities. Functionally tested in DNA amplification.

Troubleshooting Guide:

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>No amplification</td>
<td>Degraded samples</td>
<td>1. Check the integrity of DNA using agarose gel electrophoresis.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2. Use freshly prepared DNA to ensure the availability of intact template sequence for efficient amplification.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Error in protocol setup</td>
<td>Verify that the correct reagent volumes, dilutions and storage conditions have been used.</td>
</tr>
<tr>
<td>2.</td>
<td>Variability between replicates</td>
<td>Error in reaction set-up</td>
<td>Prepare large volume reaction mix, vortex thoroughly and aliquot appropriately into reaction tubes.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Air bubbles in reaction mix</td>
<td>Briefly centrifuge reaction samples/plate prior to running on a PCR machine.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pipetting error</td>
<td>Replicates can show increased variation due to poor laboratory techniques or imprecise pipettes.</td>
</tr>
<tr>
<td>3.</td>
<td>Amplification in negative control</td>
<td>Reagents contaminated</td>
<td>1. Replace all critical solutions.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2. Repeat the analysis of all tests with fresh aliquots of critical reagents.</td>
</tr>
</tbody>
</table>

Safety Information

The E. coli O157:H7 Detection Kit 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 product in the manner described in the product literature. E. coli O157:H7 Detection Kit is designed and sold for research and 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.

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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.