MBPCR005 Listeria monocytogenes Detection Kit

Description:

Listeria monocytogenes is a gram-positive intracellular organism causing severe infections that primarily affect pregnant women, newborns and immunocompromised individuals. While *Listeria spp.* are ubiquitous in nature, only *L. monocytogenes* is pathogenic to humans. In recent years, a number of outbreaks of food-borne illness involving a wide range of foods have been linked to *L. monocytogenes*. Elimination of this organism from foods is extremely difficult due to it widespread distribution and ability to grow at refrigeration temperature (4°C). The prevention of further outbreaks of listeriosis will require validation of pathogen interventions around critical control points in food processing.

NOTE: The Listeria monocytogenes Detection Kit is for *in vitro* use only.

Intended Use:

The Listeria monocytogenes Detection Kit is designed to detect the specific sequence of a virulence marker p60 encoded by **iap gene (131 bp)** for *L. monocytogenes* in various food sources, cells, environmental and clinical samples**.** Conventional PCR testing can provide rapid, sensitive and specific detection of *L. monocytogenes*. 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 Listeria monocytogenes Detection Kit is a qualitative conventional PCR kit, which allows the amplification of *Listeria monocytogenes* specific gene, **iap (131 bp)** using specific primer. The amplified target is detected 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 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 30 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.

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Features:

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

Unit Definition:

1U 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:

Components	Reagents provided for 10R (reactions)	Reagents provided for 25R (reactions)	Reagents provided for 50R (reactions)
2X PCR Master Mix **(MBT061)**	260 µl	650 µl	1.5ml
Primer Mix for *L. monocytogenes*	25 µl	60 µl	120 µl
Primer Mix for Internal Control	25 µl	60 µl	120 µl
Nuclease free water **(ML065)**	1 ml	2 ml	4 ml
6X Loading Dye **(ML015)**	100 µl	200 µl	400 µl
100 bp DNA Ladder **(MBT049)**	40 µl	90 µl	180 µl
Positive control (*L. monocytogenes* DNA)	15 µl	35 µl	65 µl
Internal Control DNA	15 µl	35 µl	65 µl

Sample Collection and Preparation:
Various food and environmental samples, clinical materials, cultured bacteria and human fecal specimens are routinely 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.

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.

Protocol:

<u>Preparation of PCR Reaction Mixture</u>

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

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In the same tube, add 2 µl of *L .monocytogenes* 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)

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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 : 95°C for 5 minutes
2. Cycling Parameters (No. of cycles: 30)
   Denaturation : 95°C for 45seconds
   Annealing : 60°C for 45 seconds
   Extension : 72°C for 30 seconds
3. Final Extension : 72°C for 8 minutes

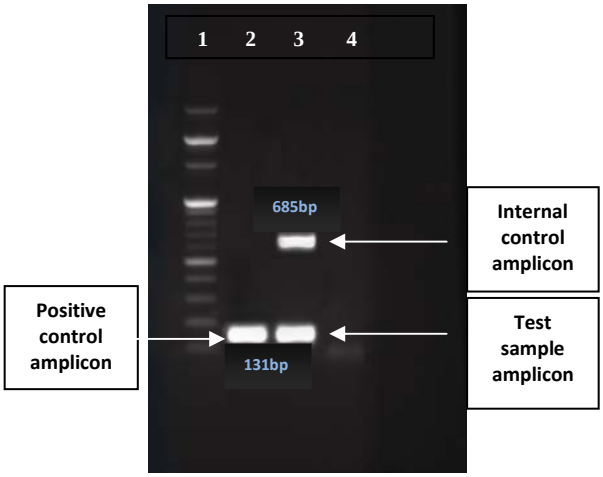
C. After amplification the products may be kept at 4°C overnight or frozen at –20°C for long-term storage.

D. Listeria PCR Assay Results Interpretation

- For analysis of the PCR data, load 10 µl of amplicon on a 2% Agarose gel along with 1µl 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 minutes
- Confirm the expected amplicon size comparing with 100 bp DNA marker



Lane no.	Samples
1	100 bp ladder
2	Positive control amplicon of *L. monocytogenes* (131bp)
3	Test sample amplicon of *L. monocytogenes* (131 bp) with internal control (685 bp)
4	Negative control

Gel image representing amplification of iap gene using target sample of *L. monocytogenes* with positive control (131bp) and internal control (685bp).

Specifications:

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

Quality Control:

Each lot of HiMedia Listeria monocytogenes Detection Kit are assayed for contaminating endonucleases, exonucleases 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 reaction mix, vortex thoroughly and aliquot appropriately into reaction tubes.
		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.
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 Listeria monocytogenes 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. Listeria monocytogenes 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.

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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