LISTERIA MONOCYTOGENES
PCR detection Kit

50 REACTIONS

Listeria monocytogenes detection kit for use with PCR Thermal Cyclers.

FOR LABORATORY USE ONLY

NAME AND INTENDED USE
Identification of Listeria monocytogenes DNA by polymerase chain reaction (PCR).

INTRODUCTION
Listeria monocytogenes is responsible of a serious food-borne disease that affects especially newborn, pregnant women, the elderly, and patients with impaired cell-mediated immunity (1). Ingestion of contaminated food causes an infection, named listeriosis, characterized by a variety of severe syndromes, such as encephalitis, meningoencephalitis, septicemia and abortion (2). Current bacterial identification methods are microbiological culture isolation or serum agglutination test. The first procedure, requiring a minimum of 5 days, is time expensive while the second is not specific because of cross-reactivity between L. monocytogenes and other Gram-positive bacteria. On the contrary PCR-based detection systems are specific and sensitive and have shortened analysis time (3). L. monocytogenes identification by PCR methods has been successfully applied to pre-enriched bacterial cultures (4), concentrated bacterial suspensions (5) or extracted food components (6).

PRODUCT DESCRIPTION
The "LISTERIA MONOCYTOGENES PCR detection Kit" allows the detection of L. monocytogenes DNA using Polymerase Chain Reaction (PCR). PCR primers specifically detect all the L. monocytogenes strains belonging to each of the three evolutionary lineages defined by Jinneman and Hill (7) The kit contains reagents and enzymes for the specific amplification of a 172 bp region of the L. monocytogenes genome. To identify possible PCR inhibition, an internal control, giving an amplicon of 112 bp, is also supplied in the PCR mix.

KIT CONTENTS
L. monocytogenes Mix: 1000 µl (2 vials)
Hot-Rescue DNA Polymerase (5U/µl): 20 µl (100 U)
L. monocytogenes Positive control: 300 µl
Water: 1000 µl
OTHER SUPPLIES REQUIRED

- Disposable latex gloves.
- Precision pipettes.
- Sterile pipette tips.
- Desktop microcentrifuge.
- Thermal cycler.
- Sterile PCR reaction tubes.
- Sterile 1.5 ml tubes.
- Sterile mineral oil (to prevent evaporation in thermal cyclers without heated lid).
- Agarose gel electrophoresis apparatus.
- DNA size standard (100 - 1000 bp).
- UV-Transilluminator.

STORAGE

Store the kit at -20°C.
Repeated thawing and freezing cycles may reduce the sensitivity and should be avoided. It is suggested freezing the reagents in aliquots for intermittently use.

GENERAL PRECAUTIONS FOR PCR

The operator should always pay attention to:
- use pipette tips with filter;
- store positive material (specimens, controls and amplicons) separately from all other reagents and, if possible, add it to the reaction mix in a facility separated space;
- do not use the same precision pipettes for reaction mix and DNA-samples;
- thaw all components samples at room temperature before starting an assay;
- when thawed, mix the components and centrifuge briefly;
- work on ice or in a cooling block.

PROTOCOL

DNA ISOLATION

Various protocols or Kits can be used to extract bacterial DNA from many food and clinical specimens or bacterial enrichment cultures.
Carry out the DNA isolation according to the procedure making sure that the extracted DNA is free from PCR inhibitors.
The protocols, tested by DIATHEVA, are available on-line in the “LIBRARY” section

For isolation of *L. monocytogenes* DNA from milk samples we recommend you to use the "LISTERIA MONOCYTOGENES DNA isolation Kit: Milk" Product Number # MBK0002

PCR SET UP

The use of thin-walled PCR tubes for all amplification steps is recommended.
Include a positive control and at least one negative control (water) in each PCR run.
Before each use thaw all reagents completely, mix and centrifuge.

The PCR assay must be prepared following the pipetting scheme below:

<table>
<thead>
<tr>
<th>FOR 1 REACTION</th>
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</thead>
<tbody>
<tr>
<td>L. monocytogenes Mix</td>
</tr>
<tr>
<td>Hot-Rescue DNA Polymerase</td>
</tr>
<tr>
<td>Sample (*Preferably = 1 µg DNA/reaction)</td>
</tr>
<tr>
<td>Total volume</td>
</tr>
</tbody>
</table>

*The sample can contain up to 1 µg L. monocytogenes DNA without inhibiting the amplification reaction.

Make a master mix combining the reagents together in a 1.5 ml tube.

Aliquot 40 µl of master mix into each PCR reaction tube before adding 10 µl sample, negative and positive control.

In order to avoid cross contamination, first pipet the negative control, then the samples and finally the positive control.

Centrifuge briefly

For PCR thermal cycler without heated lid, each reaction mixture will have to be covered with a drop of mineral oil.

**THERMAL PROFILE**

Program the PCR thermal cycler with the following parameters:

<table>
<thead>
<tr>
<th>1 cycle</th>
<th>95°C for 15 min</th>
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</thead>
<tbody>
<tr>
<td>95°C for 15 sec</td>
<td></td>
</tr>
<tr>
<td>50 cycles</td>
<td>61°C for 20 sec</td>
</tr>
<tr>
<td>72°C for 30 sec</td>
<td></td>
</tr>
<tr>
<td>1 cycle</td>
<td>72°C for 4 min</td>
</tr>
<tr>
<td>cool down to 4°C</td>
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**AGAROSE GEL ELECTROPHORESIS**

Mix 25 µl of the PCR reaction with 5 µl of DNA loading buffer. Separate the DNA in the presence of a DNA standard, on a 2% agarose gel containing ethidium bromide, for about 45-60 min. The DNA standard should be specific for the low range (100-1000 bp).
DATA ANALYSIS

The following results are possible:

<table>
<thead>
<tr>
<th>Band pattern</th>
<th>Interpretation</th>
</tr>
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<tbody>
<tr>
<td>Band at 112 bp</td>
<td>NEGATIVE SAMPLE</td>
</tr>
<tr>
<td>Band at 172 bp and possibly an additional band at 112 bp</td>
<td>POSITIVE SAMPLE</td>
</tr>
<tr>
<td>No band</td>
<td>NO DIAGNOSIS (see troubleshooting section)</td>
</tr>
</tbody>
</table>

Examples of positive and negative samples are showed in the following gel electrophoretic separation of PCR products:

Lane 1: ΦX174 DNA/BsuRI DNA molecular weight marker (MBI Fermentas)
Lane 2-5: Amplification of 50, 10, 4 and 2 Listeria monocytogenes DNA molecules.
Lane 6-7: Negative controls

TROUBLESHOOTING

<table>
<thead>
<tr>
<th>Issue</th>
<th>Solution</th>
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<tbody>
<tr>
<td>No band of 172 bp from the positive control</td>
<td>Incorrect programming of the thermal cycler: repeat the PCR with the correct settings. Degraded reagent: store PCR reagents at -20°C and keep on ice once thawed. Avoid multiple freeze-thaw cycles. Incorrect tubes: use thin-walled PCR reaction tubes.</td>
</tr>
<tr>
<td>No band of 172 bp (L. monocytogenes) and 112 bp (Internal Control) from the sample PCR</td>
<td>PCR reaction inhibition: repurify the DNA sample to remove inhibitors.</td>
</tr>
<tr>
<td>Band of 172 bp from the negative control</td>
<td>Contamination of PCR reaction: vigorous cleaning is recommended before repeat the amplification.</td>
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REFERENCES