MBPCR050  Pseudomonas aeruginosa Detection Kit

Description

*Pseudomonas aeruginosa* is a Gram-negative rod-shaped bacterium. It is one of the main causes of pneumonia in patients who are on breathing machines. It can also cause eye infections in persons using extended-wear contact lenses, urinary tract infections, skin rashes, ear infections etc. It has become an important cause of gram-negative infection, especially in patients with compromised host defense mechanisms. It is the most common pathogen isolated from patients who have been hospitalized longer than 1 week. Infections can occur in any part of the body. Symptoms depend on which part of the body is infected. Patients who are already very ill can die from a *Pseudomonas* infection.

The Pseudomonas aeruginosa Detection Kit has been developed for rapid and sensitive detection of *P. aeruginosa*.

Intended Use

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

Product characteristics

The Pseudomonas aeruginosa Detection Kit is designed for fast detection of specific sequence of 16S rRNA sequence (956bp) gene for *Pseudomonas aeruginosa* from environmental sample and clinical material samples.

**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 based on separation of DNA fragments according to their size.
Features:
- Fast and simple
- Sensitive and specific results
- Guaranteed reproducible results

Sample Source: Environmental and clinical samples

Storage and Shelf-life:
The provided kit has a shelf-life of 12 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. HiMedia does not recommend using the kit after the expiry date stated on pack.

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.

Kit Contents:
The provided PCR contains:

<table>
<thead>
<tr>
<th>Components</th>
<th>Product code</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 TaqMixture</td>
<td>MBT061</td>
<td>260 µl</td>
<td>650 µl</td>
<td>1.5ml</td>
</tr>
<tr>
<td>Primer Mix for <em>P. aeruginosa</em></td>
<td>DS0155</td>
<td>25 µl</td>
<td>60 µl</td>
<td>120 µl</td>
</tr>
<tr>
<td>Primer Mix for Internal Control (285 bp)</td>
<td>DS0223</td>
<td>25 µl</td>
<td>60 µl</td>
<td>120 µl</td>
</tr>
<tr>
<td>Molecular Biology Grade Water for PCR</td>
<td>ML065</td>
<td>1 ml</td>
<td>2 ml</td>
<td>4ml</td>
</tr>
<tr>
<td>6X Gel Loading Buffer</td>
<td>ML015</td>
<td>100 µl</td>
<td>200 µl</td>
<td>400 µl</td>
</tr>
<tr>
<td>100 bp DNA Ladder</td>
<td>MBT049</td>
<td>40 µl</td>
<td>90 µl</td>
<td>180 µl</td>
</tr>
<tr>
<td>Positive control (<em>P. aeruginosa</em> DNA)</td>
<td>DS0361</td>
<td>15 µl</td>
<td>35 µl</td>
<td>65 µl</td>
</tr>
<tr>
<td>Internal Control DNA</td>
<td>DS0123</td>
<td>15 µl</td>
<td>35 µl</td>
<td>65 µl</td>
</tr>
</tbody>
</table>

Materials needed but not provided
- Micropipette and tips
- Micro centrifuge (for spinning down the reaction mix)
- HiMedia’s HiPurA Bacterial DNA Purification Kit (MB505)
- PCR Thermal Cycler (HiMedia Code: LA948, LA949, LA950, LA1015)
Sample Material Preparation:
Various environmental samples, clinical materials, cultured bacteria and 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. The purified DNA is free of any inhibitors and can be used directly for PCR.

Enrichment of pathogens (if required):
In order to ensure sensitive detection of pathogens from different variety of samples by PCR, the pathogens need to be enriched in broth.

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 TaqMixture (MBT061) in a PCR tube

In the same tube, add 2 µl Primer Mix for *P. aeruginosa* (DS0155) + 2 µl Primer Mix for Internal Control (285 bp) (DS0223) (10 pmoles concentration provided)

Add 1-2 µl of template DNA (upto 50 ng of extracted DNA) and add 1µl of Internal Control DNA (DS0123) (provided)

Add Molecular Biology Grade Water for PCR (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 5 minutes

2. Cycling Parameters (No. of cycles: 30)
   - Denaturation : 94°C for 30 seconds
   - Annealing : 58°C for 30 seconds
   - Extension : 72°C for 30 seconds

3. Final Extension : 72°C for 5 minutes

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

D) PCR sample Assay Results Interpretation

- For analysis of the PCR data, load 5-8 µl of amplicon on a 1% Agarose gel along with 1 µl of 6X Gel Loading Buffer (ML015) (provided).
- Load 3 µl of 100 bp DNA ladder (MBT049) (provided) in separate well.

E) Ethidium bromide (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

Warning
Certified for in vitro Diagnostic Use (IVD). Not for Medicinal Use.

Precautions
Read the procedure carefully before starting the experiment.

Performance and Evaluation
Each lot of HiMedia’s Pseudomonas aeruginosa Detection Kit is tested against predetermined specifications to ensure consistent product quality.

Quality Control
Each lot of HiMedia’s Pseudomonas aeruginosa Detection Kit is assayed for contaminating endonuclease, exonuclease and non-specific DNase activities. Functionally tested in DNA amplification.
Gel image representing amplification of 16S rRNA sequence gene amplicon (956bp) of template *Pseudomonas aeruginosa* with positive control and Internal Control (285 bp)

**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>

<table>
<thead>
<tr>
<th>Lane no.</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>100 bp ladder</td>
</tr>
<tr>
<td>1</td>
<td>Positive control amplicon <em>Pseudomonas aeruginosa</em> (956bp) with internal control (285bp)</td>
</tr>
<tr>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

Please refer disclaimer Overleaf.
Safety information

The Pseudomonas aeruginosa 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. Pseudomonas aeruginosa 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.