16S rRNA PCR Kit (Real-Time PCR)

Description
Clinical microbiology laboratory is responsible for the isolation or detection of microorganisms to establish the diagnosis of infection. Microorganisms are typically identified by morphological, physiological, and biochemical characteristics, among other attributes. However, procedures for characterizing these features are time-consuming and sometimes yield incorrect or no results. DNA analysis has been increasingly used for microorganism identification. The analysis of the nucleotide sequences of the 16S ribosomal RNA gene has emerged as the single best method to identify bacteria.

NOTE: HiMedia’s 16S rRNA PCR Kit (Real-time PCR) is for in-vitro use only.

Intended Use
Recommended for sensitive detection of any bacterial species.

Principle
Real-time Polymerase Chain Reaction, also called quantitative Polymerase Chain Reaction (qPCR) or kinetic Polymerase Chain Reaction, is a laboratory technique based on the principle of Polymerase Chain Reaction. This technique is used to amplify and simultaneously quantitate a targeted DNA sequence. Real-time PCR systems based on SYBr Green assays have increasingly been used for accurate, reliable detection and quantitation of various food-borne pathogens. HiMedia’s 16S rRNA PCR Kit (Real-time PCR) is designed to specifically amplify a region within the bacterial 16S rRNA.

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.

Diagrammatic representation of preferential binding of SYBr Green Dye to specific DNA fragments in Real-Time PCR

The SYBr Green dye cycles between an unbound (Denaturation step) and a bound (Annealing through Extension) state as the reaction progresses. Signal intensity increases as the quantity of amplicons increase in later cycles indicating amplification. During elongation, more and more dye molecules bind to the newly synthesized DNA. If the reaction is monitored continuously, an increase in fluorescence is viewed in real-time. Upon denaturation of the DNA for the next heating cycle, the dye molecules are released and the fluorescence signal falls.
Features
- Fast and simple
- Sensitive and specific results
- Guaranteed reproducible results
- Rapid detection of all relevant clinical pathogens

Sample Source: Bacterial / Clinical / Food samples

Storage
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.

Kit Contents:
The provided PCR kit contains:

<table>
<thead>
<tr>
<th>Components</th>
<th>Product Code</th>
<th>Reagents provided (reactions)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hi-SYBr Master Mix (with Taq Polymerase)</td>
<td>MBT074</td>
<td>150 µL 375 µL 750 µL</td>
</tr>
<tr>
<td>16S Primer Mix</td>
<td>DS0299</td>
<td>25 µL 62.5 µL 125 µL</td>
</tr>
<tr>
<td>Positive control (Bacterial DNA)</td>
<td>DS0122C</td>
<td>15 µL 37.5 µL 75 µL</td>
</tr>
<tr>
<td>Molecular Biology Grade Water for PCR</td>
<td>ML065</td>
<td>500 µL 1.25 mL 2.5 mL</td>
</tr>
</tbody>
</table>

*For a 20 µL PCR reaction

Materials needed but not provided
- PCR tubes (Product code PW1255) or PCR Strips (Product code: PR17) or PCR Plates (Product code: PR2 / PR3 / PR19)
- Insta Q Real Time PCR System (Product Code: LA1012 / LA1023 / LA1024)
- Barrier Micropipette Tips (LA749 / LA750 / LA751 / LA859)
- Micropipettes

General Preparation Instructions
- Before use all PCR components should be completely thawed on ice (4°C).
- Perform the amplification reactions in a clean area, preferably in a biosafety cabinet.
- Use of aerosol barrier pipette tips is recommended to reduce contamination risks from extraneous DNA templates.
- Extract and store positive control sample (if used) separately from all other reagents to avoid contamination and add it to the reaction mix in a separate area.

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 clinical and environmental samples and cultured bacteria are routinely examined. For extraction and purification of pure bacterial DNA for high yield, perform the nucleic acid purification using HiMedia’s HiPurA™ Bacterial Genomic DNA Purification Kit (MB505) as instructed in the protocol.
A. Protocol for PCR Master Mix Preparation

<table>
<thead>
<tr>
<th>Components</th>
<th>Recommended volume to be added per reaction (for a 20 µL reaction)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hi-SYBr Master Mix (with Taq Polymerase) <em>(MBT074)</em></td>
<td>10 µL</td>
</tr>
<tr>
<td>16S Primer Mix</td>
<td>2 µL</td>
</tr>
<tr>
<td>Template DNA</td>
<td>5 µL</td>
</tr>
<tr>
<td>Molecular Biology Grade Water for PCR</td>
<td>Up to 20 µL</td>
</tr>
<tr>
<td>Total Volume</td>
<td>20 µL</td>
</tr>
</tbody>
</table>

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

Centrifuge the tube briefly at 6000 rpm for about 10 seconds. Place the tubes in real-time PCR machine and set the recommended PCR program (mentioned below). Interpret the data from the amplification plot (observe the Ct values).

B. Recommended PCR program

1. Initial denaturation : 94°C for 10 minutes No. of cycles: 1
2. Denaturation : 94°C for 30 seconds
3. Annealing (Plate Read) : 56°C for 30 seconds No. of cycles: 40
4. Extension (Plate Read) : 72°C for 45 seconds
5. Melt Curve Analysis as per HiMedia’s Insta Q96 Real-Time PCR Machine
   a. 95°C : 15 seconds
   b. 60°C : 1 minute
   c. 95°C : 15 seconds
   d. Increment : 0.5°C
   e. On Hold : 10 seconds
6. Hold : 4°C for ∞

**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 16S rRNA PCR Kit (Real-time PCR) is tested against predetermined specifications to ensure consistent product quality.

**Quality Control**

Each lot of HiMedia’s 16S rRNA PCR Kit (Real-time PCR) is assayed for contaminating endonuclease, exonuclease and non-specific DNase activities. Functionally tested in DNA amplification.

**Amplification Data**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Sample</th>
<th>C&lt;sub&gt;t&lt;/sub&gt; value</th>
<th>Tm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Negative control</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>2</td>
<td>Bacterial Template DNA</td>
<td>10.62</td>
<td>89.9</td>
</tr>
</tbody>
</table>

Figure: Data representing real-time amplification data of Bacterial Template DNA with C<sub>t</sub> values (provided in table)
<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 master mix, vortex thoroughly and aliquot 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 real-time PCR instrument.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pipetting error</td>
<td>Ct values of replicates can show increased variation due to poor laboratory technique 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>

Please refer disclaimer Overleaf.
Safety Information
HiMedia’s 16S rRNA PCR Kit (Real-time PCR) 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.

Storage temperature
-20°C
-10°C

Do not use if package is damaged

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Disclaimer:
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