MBPCR094 Hi-PCR® 16S rRNA Semi-Q PCR Kit

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 Hi-PCR® 16S rRNA Semi-Q PCR Kit is for *in vitro* use only.

Intended Use: HiMedia's Hi-PCR® 16S rRNA Semi-Q PCR Kit is designed for the detection of a specific sequence of the **16S rRNA gene** in bacteria from various sources.

Principle:
HiMedia's Hi-PCR® 16S rRNA Semi-Q PCR Kit is designed for the detection of a specific sequence of the **16S rRNA gene** in bacteria from various food sources, cells, clinical samples, etc**.** The bacterial 16S rRNA specific primers generate an amplicon of **1.5 kb.** This kit also contains **Positive control.**

Positive control: This is a control reaction using a known template. 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). The 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

ISO 9001-2015 CERTIFIED
WHO GMP CERTIFIED
ISO 13485-2016 CERTIFIED

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Sample Source: Various food, clinical and environmental samples as well as cultured bacteria are routinely examined.

Storage:

The provided kit has a shelf-life of 12 months when stored between -10°C to -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 contains:

Components	Product code	Reagents provided for (reactions)*	
		25R	50R
2X PCR TaqMixture	MBT061	675 µL	1.35 mL
16S Primer Mix	DS0299	54 µL	108 µL
Positive control (Bacterial DNA)	DS0122C	5 µL	10 µL
Molecular Biology Grade Water for PCR	ML065	600 µL	1.2 mL

***** For a 50µl PCR reaction

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:

For extraction and purification of high yield and pure bacterial DNA, perform the nucleic acid purification using HiMedia's HiPurA® Bacterial Genomic DNA Purification Kit (MB505) as instructed in the protocol.

Materials needed but not provided:

- PCR tubes (Product code PW1255) or PCR Strips (Product code: PR17) or PCR Plates (Product code: PR2 / PR3 / PR19)
- Thermal Cycler (Product Code: LA948/LA949/LA950/LA1006/LA1015/LA1059/LA1060/LA1066)
- Barrier Micropipette Tips (Product Code: LA749 / LA749A / LA751 / LA751A / LA750 / LA750A / LA859 / LA859A)
- Micropipettes

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.

A. Protocol for PCR Master Mix Preparation:

Perform PCR reactions for each DNA sample as per the following table:

Components	Recommended volume to be added per reaction
2X PCR TaqMixture **(MBT061)**	25 µL
16S Primer Mix **(DS0299)**	2 µL
Template DNA	2 µL
Molecular Biology Grade Water for PCR	Up to 50 µL

NOTE: (Optional) – The user can also set up an additional PCR reaction containing 1µL of Positive control DNA (provided) in a separate tube.

Centrifuge the tube briefly at 6000 rpm for about 10 seconds and place the tubes in the PCR machine and set the recommended PCR program (mentioned below). Interpret the data using Agarose Gel Electrophoresis.

B. Recommended PCR program:

1	Initial denaturation	:	94°C for 05 minutes	No. of cycles: 1
2	Denaturation	:	94°C for 30 seconds	No. of cycles: 30
3	Annealing	:	60°C for 30 seconds	
4	Extension	:	72°C for 45 seconds	
5	Final Extension	:	72°C for 5 minutes	No. of cycles: 1

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

16S 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 Gel Loading Buffer (ML015).
- Load 4 µl of 100 bp DNA ladder (MBT049) in separate well.

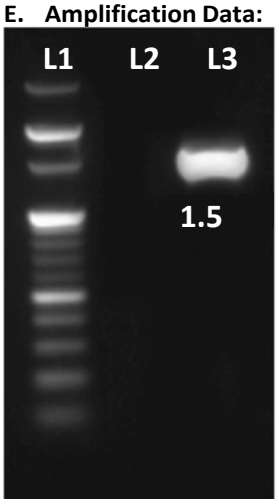
D. EtBr-staining to check results:

- 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.

E. Quality Control:

Each lot of HiMedia's Hi-PCR® 16S rRNA Semi-Q PCR Kit is assayed for contaminating endonuclease, exonuclease and non-specific DNase activities. Functionally tested in DNA amplification.

E. Amplification Data:



L1 – 100 bp ladder
L2 – Negative Control
L3 – Positive control for 16S gene (1.5 kb)

Gel image representing amplification Data of 16S gene (1.5 kb)

Precautions

Read the procedure carefully before beginning the protocol. Wear protective gloves/protective clothing/eye protection/face protection. Follow good clinical laboratory practices while handling clinical samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred in safety data sheets of the product.

Performance and Evaluation

Each lot of HiMedia's Hi-PCR® 16S rRNA Semi-Q PCR Kit is tested against predetermined specifications to ensure consistent product quality.

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

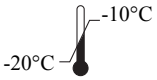
The Hi-PCR® 16S rRNA Semi-Q PCR Kit is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

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

Do not use if package is damaged

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