MBPCR087 Hi-PCR® 16S rRNA SYBr 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 SYBr PCR Kit is for *in-vitro* use only.

Intended Use

Recommended for sensitive detection of any bacterial species.

Principle

HiMedia's Hi-PCR® 16S rRNA SYBr 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 (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.

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.

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WHO GMP CERTIFIED
ISO 13485-2016 CERTIFIED

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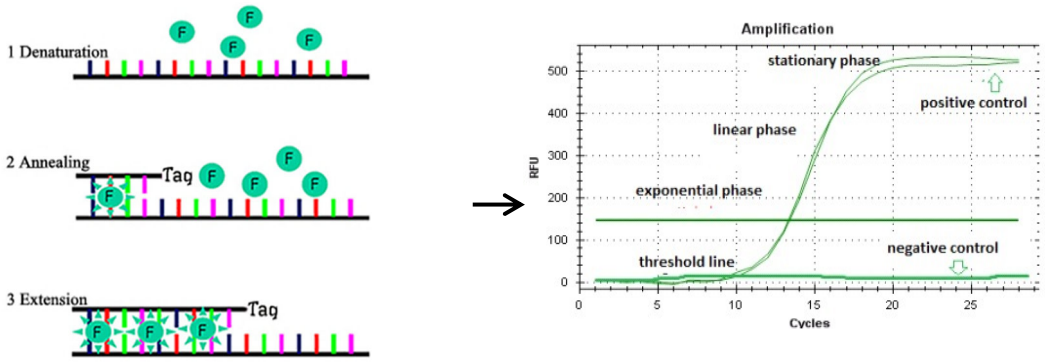
Diagrammatic representation of SYBr Green Chemistry in Real-Time PCR



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

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 kit contains:

Components	Product code	Reagents provided for (reactions)*	
		25R	50R
Hi-SYBr Master Mix (with Taq Polymerase)	MBT074	338 µl	675 µl
16S rRNA Primer Mix	DS0299	27 µl	54 µl
Positive control (Bacterial DNA)	DS0122C	5 µl	10 µl
Molecular Biology Grade Water for PCR	ML065	290 µl	580 µL

***** For a 25µl 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

Various clinical 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.

Materials needed but not provided:

- PCR tubes (Product code PW1255) or PCR Strips (Product code: PR17) or PCR Plates (Product code: PR2 / PR3 / PR19) & Sealing film (PR18)
- Insta Q Real Time PCR System (Product Code: LA1012/LA1073/LA1023/LA1024/LA1074)
- Barrier Micropipette Tips (Product Code: LA749 / LA749A / LA751 / LA751A / LA750 / LA750A / LA859 / LA859A)
- 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.

A. Protocol for PCR Master Mix Preparation

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

Components	Product code	Recommended volume to be added per reaction (µL)
Hi-SYBr Master Mix (with Taq Polymerase)	MBT074	12.5 µL
16S rRNA Primer Mix	DS0299	1 µL
Template DNA	-	2 µL
Molecular Biology Grade Water for PCR	ML065	Up to 25 µL

NOTE: (Optional) – The user can also set up an additional PCR reaction containing 1µL of Positive control DNA (DS0122A) (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

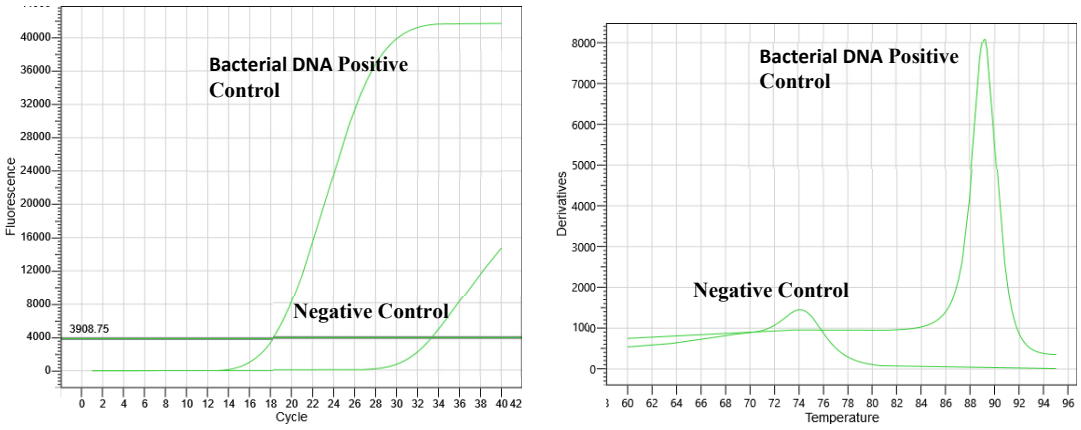
1.	Initial denaturation	:	94°C for 5 minutes	No. of cycles: 1
2.	Denaturation	:	94°C for 30 seconds	No. of cycles: 40
3.	Annealing (Plate Read)	:	60°C for 30 seconds	
4.	Melt Curve Analysis as per HiMedia's Insta Q96 Real-Time PCR Machine			
	95°C	:	15 seconds	
	60°C	:	1 minute	
	95°C	:	15 seconds	
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

NOTE: The user can also set up a melt curve program as per their existing PCR instrument.

C. Quality Control

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

D. Amplification Data Amplification plot **Melt curve**



Sr. No.	Sample	C_t value	Tm (°C)
1	Positive control	18.3	89.1
2	Negative control	33.4	74.4

Figure: Data representing real-time amplification of Bacterial Template DNA with C_t values (provided in table)

Data Interpretation

Melting Temperature (Tm)*	Result Interpretation
88°C-91°C	Positive for16S rRNA gene

*** Tm values can slightly vary for different sample types. If the Tm values show significant variation from those mentioned in the above table, then the sample is considered to be negative for 16s rRNA.**

Precautions

Read the procedure carefully before starting the experiment. 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 SYBr 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 master mix, vortex thoroughly and aliquot into reaction tubes.
		Air bubbles in reaction mix	Briefly centrifuge reaction samples/plate prior to running on a real-time PCR instrument.
		Pipetting error	C_t values of replicates can show increased variation due to poor laboratory technique 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

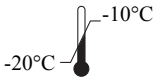
HiMedia's Hi-PCR® 16S rRNA SYBr PCR Kit is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

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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