

MBPCR015

Mycoplasma Detection Kit (Real-Time)

Description:

Cell culture is a critical technique in biological and medical research. Contamination of cell cultures can profoundly affect research results by decreasing cell growth, altering phenotypic and metabolic characteristics, inhibiting differentiation and cell death. *Mycoplasma spp.* is the most common contaminant detected in cell cultures.

Mycoplasma contamination of cell lines is one of the major problems in cell culturing. About 15-35% of all cell lines are infected with a limited number of mycoplasma species of predominantly human, swine or bovine origin. PCR represents a sensitive, specific, accurate, inexpensive and quick mycoplasma detection assay that is suitable for the routine screening of cell cultures.

Mycoplasmas belong to the class Mollicutes, which includes over 100 species of *Mycoplasma* and the very closely related genus, *Acholeplasma*. The most prevalent *Mycoplasma* and *Acholeplasma* contaminants of cultured cells are *Mycoplasma orale*, *Mycoplasma hyorhinitis*, *Mycoplasma arginini*, *Mycoplasma fermentans*, *Mycoplasma hominis* and *Acholeplasma laidlawii*.

Among bacterial infections, *Mycoplasma* contamination poses a particular challenge because it does not conspicuously overgrow the cell culture, can only be detected using special assays, and is resistant to many commonly used antibiotics. Thus, contaminations may be insidious allowing continued undetected propagation of *Mycoplasma*, which is then spread to other cultures within the laboratory, and may be further disseminated by interchange of cell lines among laboratories.

NOTE: The Mycoplasma Detection Kit (Real- Time) is for *in vitro* use only.

Principle:

The Mycoplasma Detection Kit (Real-time) is a quantitative *in vitro* test designed for amplification of targeted gene with specifically designed primers. This PCR contains a set of primers targeting the generic GPO3 and MGSO (270 bp) for pathogenic *Mycoplasma spp.*

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 Mycoplasma Detection Kit (Real-time), is one such SYBr green based qPCR technique which allows amplification of GPO3 and MGSO gene.

Registered Office :

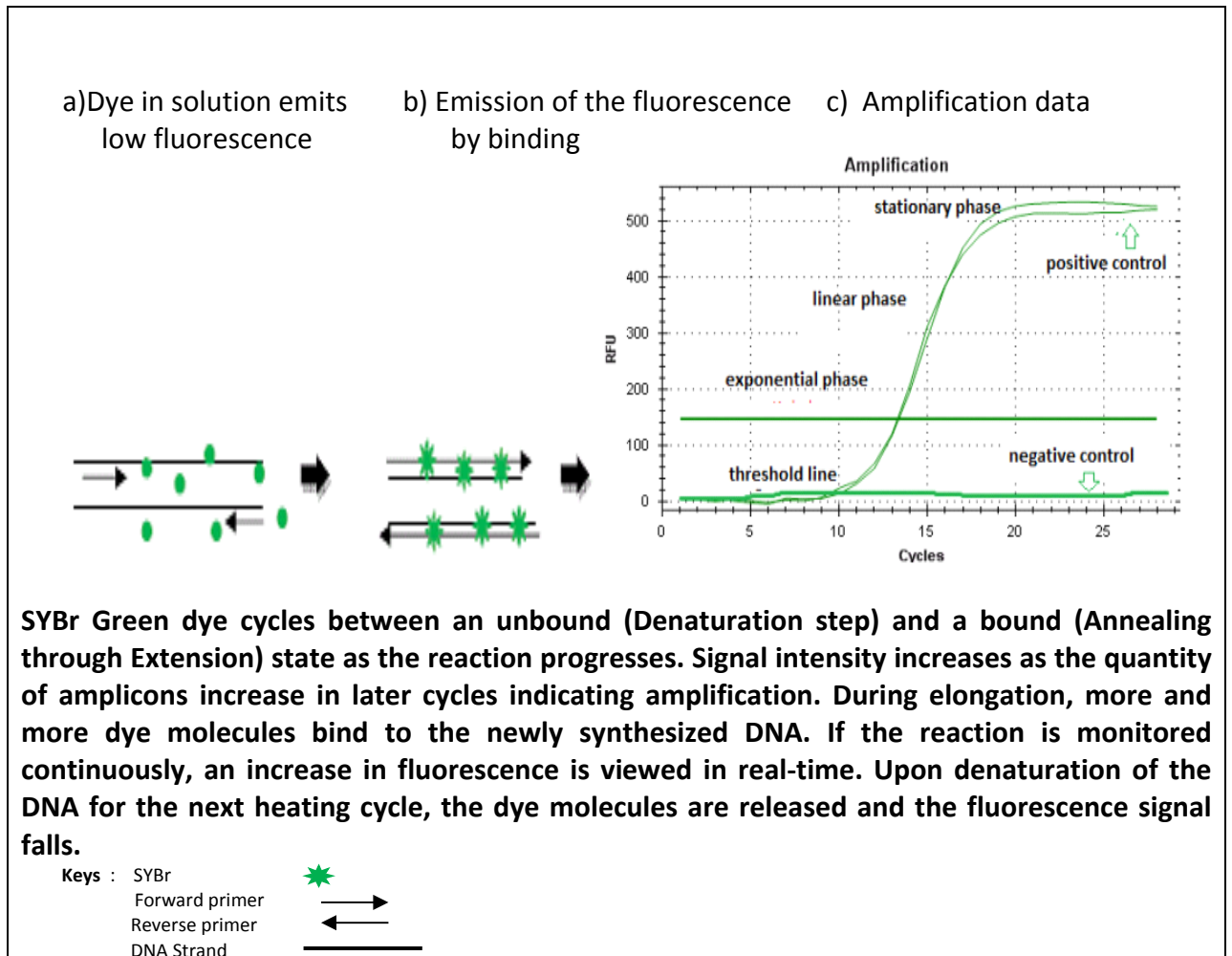
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A) Diagrammatic representation of preferential binding of SYBr Green Dye to specific DNA fragments in real-time PCR.



Features:

- Fast and simple
- Sensitive and specific results
- Guaranteed reproducible results
- Rapid detection of all relevant clinical pathogens

Kit Contents:

The provided PCR kit contains:

Components	Reagents provided for 10R (reactions)*	Reagents provided for 25R (reactions)*	Reagents provided for 50R (reactions)*
Hi-SYBr master mix (2X master mix containing SYBr Green, Assay buffer, Taq Polymerase, MgCl ₂ , dNTPs) (MBT074)	150 µl	400 µl	700 µl
Primer Mix	25 µl	60 µl	120 µl
Nuclease free water (ML065)	1 ml	2 ml	4 ml

* For a 20µl PCR reaction

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.

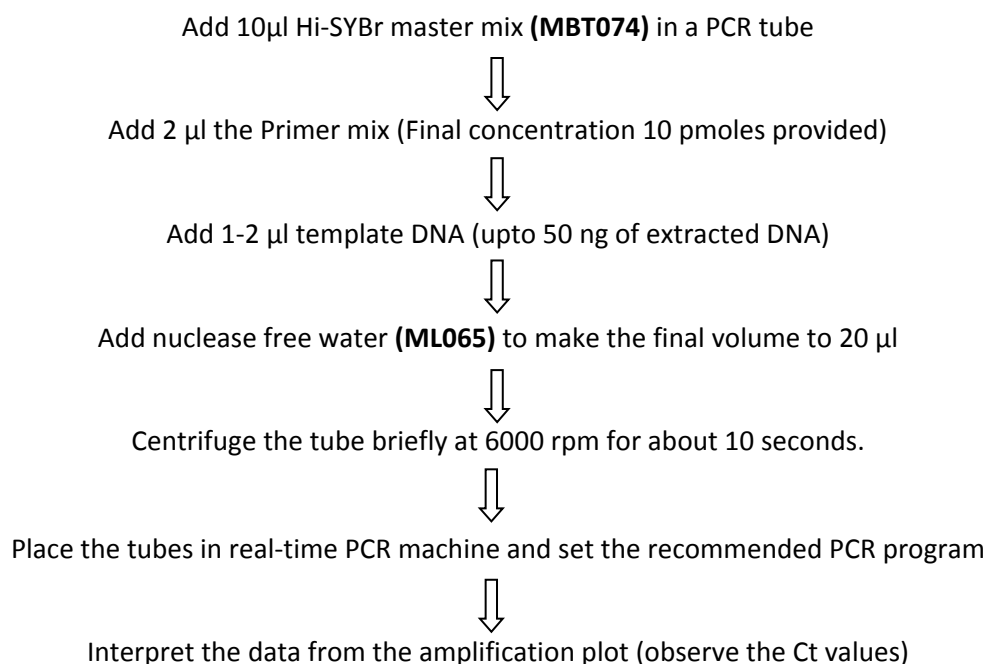
Sampling and Handling:

Sample Preparation:

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

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.

Flow Chart for setting up PCR Reaction



Recommended PCR program:

- Initial denaturation : 95°C for 10 minutes
- Cycling Parameters (No. of cycles: 35)
 - Denaturation : 95°C for 30 seconds
 - Annealing : 55°C for 30 seconds
 - Extension : 72°C for 30 seconds
- Final Extension : 72°C for 10 minutes.

Amplification Data:

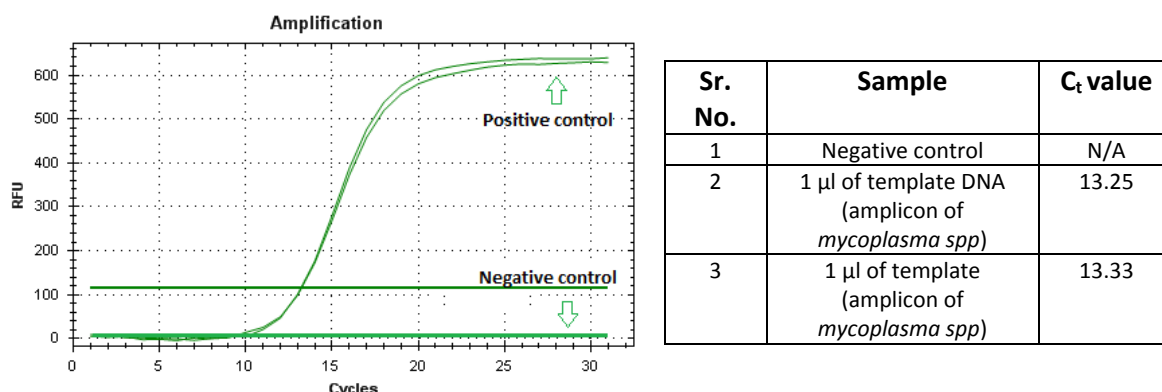


Figure: Data representing real-time amplification data of *Mycoplasma* with C_t values (provided in table)

Sensitivity: Detectable upto 100-1000 CFU / ml (mg).

Storage:

The provided kit has a shelf-life of 6 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.

Quality Control:

Each lot of HiMedia's Mycoplasma Detection Kit (Real-time) is assayed for contaminating endonuclease, exonuclease and non-specific DNase activities. Functionally tested in DNA amplification.

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	<ol style="list-style-type: none"> 1. Replace all critical solutions 2. Repeat the analysis of all tests with fresh aliquots of critical reagents.

Safety Information

The Mycoplasma Detection Kit (Real-time) 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 Mycoplasma Detection Kit (Real-time) in the manner described in the product literature. The kit is designed, sold for research and for *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.

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