

**MBPCR122**

**Hi-PCR<sup>®</sup> Mycoplasma gallisepticum SYBr PCR Kit**

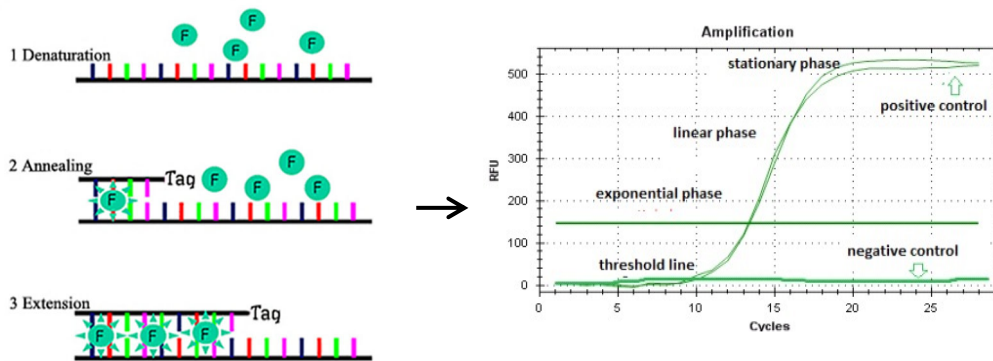
**Description**

*Mycoplasma gallisepticum* (MG) infection is one of the most significant pathogens of poultry with a worldwide distribution. Mycoplasmas are minute and bacteria like minute organisms without cell walls. Strains of *M. gallisepticum* differ greatly from other Mycoplasmas in terms of pathogenicity, infectivity and transmissibility. The infection may have diverse clinical manifestations; mainly being respiratory diseases and chicken embryo mortality. Molecular advances in detection of *M. gallisepticum* have proven successful in minimizing the heavy losses occur that used to occur during international trade in the commercial poultry industry. Hence PCR based diagnostic techniques provide for a rapid and sensitive alternative to traditional methods which require time-consuming procedures.

**NOTE: HiMedia’s Hi-PCR<sup>®</sup> Mycoplasma gallisepticum SYBr PCR Kit is for *in-vitro* use only.**

**Principle**

Real-time PCR also called quantitative PCR (qPCR) or kinetic PCR, is a laboratory technique based on the principle of PCR. This technique is used to amplify and simultaneously quantitate a targeted DNA sequence. The presence of SYBr Green Dye a dsDNA-binding dye in the Hi-SYBr Master Mix allows for simplified assay design without the need for additional fluorescent probes and enables assay verification using a melt-curve analysis. HiMedia’s Hi-PCR<sup>®</sup> Mycoplasma gallisepticum SYBr PCR Kit is a qualitative Real-Time PCR kit which includes the amplification of 16S rRNA gene (590 bp) of *M. gallisepticum*.



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.

**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.

## Features

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

## 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 the sensitivity of the assay. HiMedia does not recommend using the kit after the expiry date stated on the pack.

## Kit Contents

The provided PCR Kit contains:

Components	Product code	Reagents provided for (reactions)*	
		25R	50R
Hi-SYBr master mix	MBT074	270	540
Primer Mix for <i>M. gallisepticum</i>	DS0240	54	108
Positive Control for <i>M. gallisepticum</i>	DS0269	25	50
Molecular Biology Grade water for PCR	ML065	100	200

\*For a 20 µL 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 to individual safety data sheets.

## General Preparation Instructions

- Before use, a 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 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 (µL)
Hi-SYBr master mix	10
<i>M. gallisepticum</i> Primer Mix	2
Template DNA / Negative Control	5
Molecular Biology Grade water for PCR	Up to 20

**NOTE: For a Positive Control reaction tube, 5µL of positive control DNA is to be added along with above mentioned components instead of template (extracted DNA).**

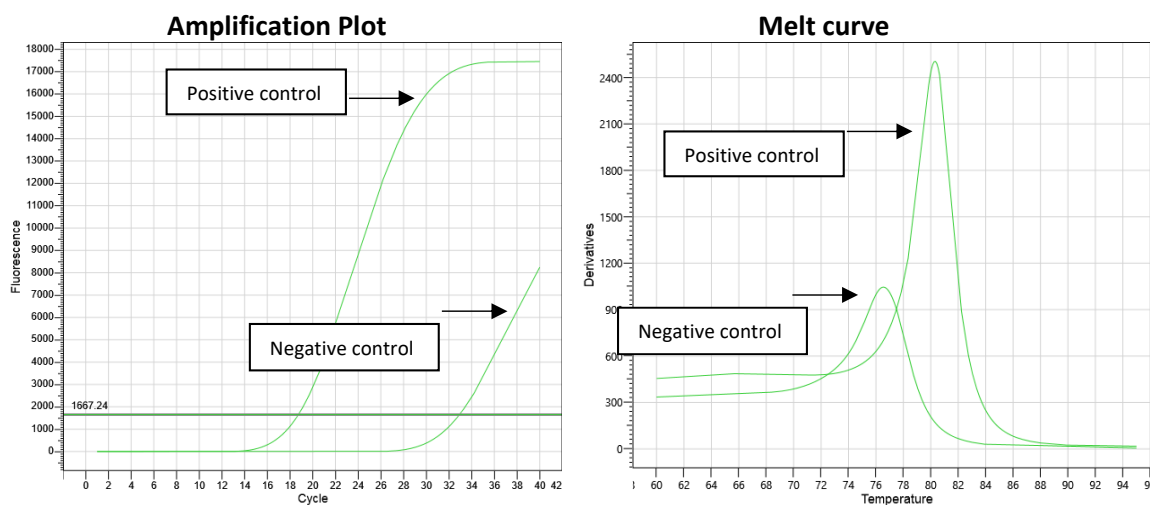
Centrifuge the tube briefly at 6000 rpm for about 10 seconds and place the tubes in the PCR machine and set the recommended PCR program (mentioned below).

**B. Recommended PCR program**

1. Initial denaturation : 94°C for 5 minutes      No. of cycles: 1
2. Denaturation : 94°C for 30 seconds
3. Annealing (Plate Read) : 58°C for 30 seconds } No. of cycles: 40
4. 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

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

**C. Amplification data**



Sr. No.	Sample	C <sub>t</sub> value	T <sub>m</sub> Value
1	Positive control	18.4	80.3
2	Negative control	32.5	76.6

Image representing real-time amplification data of *Mycoplasma gallisepticum* (Ct and Tm values provided in table)

**Data Interpretation**

Melting Temperature (T <sub>m</sub> )*	Result Interpretation
78°C-82°C	Positive for <i>Mycoplasma gallisepticum</i>

\* T<sub>m</sub> values can slightly vary for different sample types. If the T<sub>m</sub> values show significant variation from those mentioned in the above table, then the sample is considered to be negative for *Mycoplasma gallisepticum*.

**Precautions**

Read the procedure carefully before starting the experiment.

**Performance and Evaluation**

Each lot of Hi-PCR® *Mycoplasma gallisepticum* SYBr PCR Kit is tested against predetermined specifications to ensure consistent product quality.

**Quality Control**

Each lot of HiMedia's Hi-PCR® Mycoplasma gallisepticum SYBr PCR Kit is assayed for contaminating endonuclease, exonuclease and non-specific RNase / DNase activities. Functionally tested for 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 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

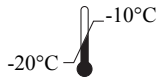
HiMedia's Hi-PCR® Mycoplasma gallisepticum SYBr PCR Kit is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

### Disposal

The 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 of 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](mailto:mb@himedialabs.com).



Storage temperature



Do not use if package is damaged



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