

## MBPCR167

## Hi-PCR<sup>®</sup> Mycoplasma gallisepticum Probe 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:** Hi-PCR<sup>®</sup> Mycoplasma gallisepticum Probe PCR Kit is for *in-vitro* use only.

### Intended Use

Recommended for sensitive and specific detection of *M. gallisepticum* in animal samples.

### 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 PCR. This technique is used to amplify a targeted DNA sequence by use of hydrolysis probes that are short oligonucleotides that have a fluorescent reporter dye attached to the 5' end and a quencher dye to the 3' end. Hi-PCR<sup>®</sup> Mycoplasma gallisepticum Probe PCR Kit is designed to detect the **cytadhesin encoding surface protein (*mgc2*) gene of *M. gallisepticum* in FAM channel** and the **Internal Control in JOE channel** in a single tube reaction. The kit allows sensitive and specific detection of *M. gallisepticum* in a single tube reaction.

### Negative Control

A negative control is needed to ensure that the reagents, equipment, and environment used in the assay is not contaminated. In this reaction, nuclease free water is used as the template.

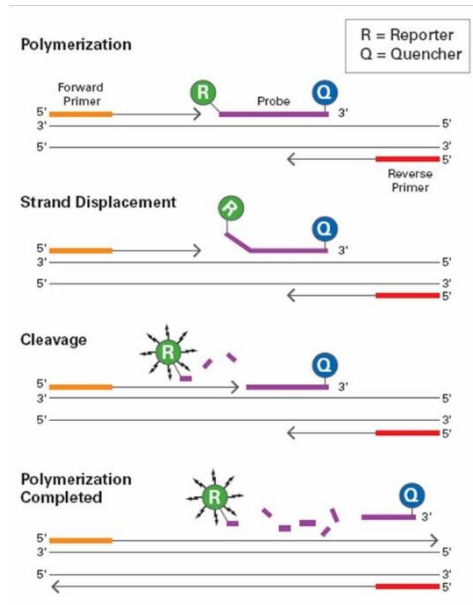
### Positive control

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.

### Internal Control

This is a control sequence which is amplified in the same reaction tube along with the target sequence (target species) but detected with a different primer (i.e. Multiplex PCR). An internal control is often used to detect the failure of amplification in cases where the target sequence is not amplified.

## Diagrammatic representation of preferential binding of probe specific to DNA fragments in Real-time PCR



**Polymerization:** A fluorescent reporter (R) dye and a quencher (Q) are attached to the 5' and 3' end of the probe respectively

**Strand displacement:** When the probe is intact, the report dye emission is quenched.

**Cleavage:** During each extension cycle, the DNA polymerase cleaves the reporter dye from the probe

**Polymerization completed:** Once separated from the quencher, the reporter dye emits its characteristic fluorescence

While the probe is intact, the proximity of the quencher dye greatly reduces the fluorescence emitted by the reporter dye by fluorescence resonance energy transfer (FRET). The probes are designed such that they anneal within a DNA region amplified by a specific set of primers. During PCR amplification, these probes will hybridize to the target sequences located in the amplicon i.e. the DNA. As the *Taq* DNA polymerase replicates the template with the bound probe, the 5'-nuclease activity of the polymerase enzyme cleaves the fluorescent probe. The end result in cleavage of the probe is separation of the reporter dye from the quencher dye and increasing the reporter dye signal. As the probe is removed from the target strand, primer extension continues to the end of the template strand. Hence, fluorescence detected in the quantitative PCR thermal cyclers is directly proportional to the fluorophore released and the amount of DNA template present in the PCR. Thus, inclusion of the probe does not inhibit the overall PCR process.

### Features

- Fast and simple
- Good sensitivity and specific results
- Guaranteed reproducible results

**Types of Specimen:** Bacterial culture / Blood sample

### 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.

### Storage and Shelf life

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 the 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 Laboratories 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)* (µL) |     |
|--|--------------|---|-----|
|  |              | 25R                                     | 50R |
| Hi-Quanti 2X Realtime PCR Master Mix     | MBT180       | 338                                     | 675 |
| <i>M. gallisepticum</i> Primer-Probe Mix | DS0419A      | 27                                      | 54  |
| Internal Control Primer-Probe Mix        | DS1021       | 27                                      | 54  |
| Internal Control DNA                     | DS1022       | 27                                      | 54  |
| <i>M. gallisepticum</i> Positive Control | DS0269A      | 25                                      | 50  |
| Molecular Biology Grade Water for PCR    | ML065        | 150                                     | 300 |

**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 / LA1023 / LA1024 / LA1073 / LA1074)
- Barrier Micropipette Tips (Product Code: LA749 / LA749A / LA751 / LA751A / LA750 / LA750A / LA859 / LA859A)
- Micropipettes
- HiPurA™ Multi-Sample DNA Purification Kit (MB554)

**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**

| Components  | Product code | Volume to be added for 1R (for a 25 µL reaction) |
|---|--------------|--|
| 2X PCR TaqMixture   | MBT180       | 12.5   |
| <i>M. gallisepticum</i> Primer-Probe Mix  | DS0419A      | 1.0  |
| Internal Control Primer-Probe Mix   | DS1021       | 1.0  |
| Internal Control DNA  | DS1022       | 1.0  |
| Molecular Biology Grade Water for PCR   | ML065        | 4.5  |
| Test – Extracted Sample DNA<br>Positive Control – Provided in Kit (DS0269A)<br>Negative Control - Water | -            | 5.0  |
| Total volume  | -            | <b>25</b>  |

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 | : 95°C for 10 minutes              | } No. of cycles: 40 |
| 2. Denaturation         | : 95°C for 15 seconds              |                     |
| 3. Annealing            | : 50°C for 20 seconds (Plate Read) |                     |
| 4. Plate Read           | : FAM/JOE                          |                     |

#for instruments not calibrated for JOE, HEX can be used

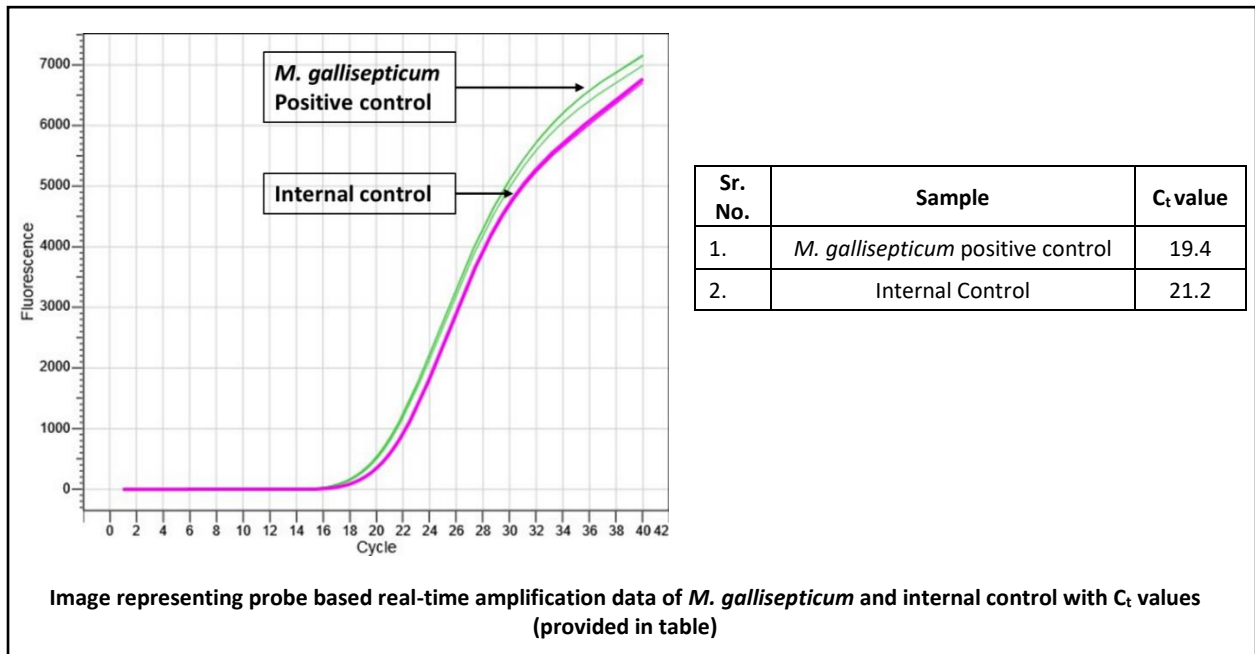
## Data Analysis

The following conditions should be met for a valid diagnostic test:

| Control          | Detection channel               |                        |
|------------------|---------------------------------|------------------------|
|                  | FAM ( <i>M. gallisepticum</i> ) | JOE (Internal Control) |
| Positive Control | +                               | +/-*                   |
| Negative Control | -                               | +                      |

\*The presence or absence of a signal in the JOE channel is not relevant for the validity of the test run due to competition between the test template and Internal Control template.

## Amplification Data



## Data Interpretation

| Detection Channel               |                        | Result Interpretation  |
|---------------------------------|------------------------|--|
| FAM ( <i>M. gallisepticum</i> ) | JOE (Internal Control) |  |
| +                               | +                      | Positive for <i>M. gallisepticum</i>   |
| -                               | +                      | Negative for <i>M. gallisepticum</i>   |
| -                               | -                      | PCR inhibition or reagent failure.<br>Repeat PCR or repeat extraction from original sample |

| Ct value    | Result           |
|-------------|------------------|
| ≤ 35        | Detected (+)     |
| > 35 or N/A | Not detected (-) |

## Warning and 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.

### Limitations

Although rare, mutations within the highly conserved regions of the targets genes covered by the kit's primers and/or probe may result in under quantitation or failure to detect the presence of the target regions in these cases. Validity and performance of the assay design are revised at regular intervals.

### Performance and Evaluation

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

### Analytical Sensitivity - Limit of Detection (LoD)

The Limit of Detection (LoD) is defined as the concentration (copies per  $\mu\text{L}$  of the eluate) of target molecule that can be detected at 95% or greater probability. The LoD assay for the Hi-PCR® Mycoplasma gallisepticum Probe PCR Kit was performed using Synthetic DNA by preliminary testing a 10-fold dilution series in triplicates per concentration followed by confirmation with 20 replicates of the concentration determined to be the LoD. The detectable Limit of Detection (LoD) of *M. gallisepticum* was determined to be **25 copies/ $\mu\text{L}$** .

### Quality Control

Each lot of Hi-PCR® Mycoplasma gallisepticum Probe PCR Kit 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.<br>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 a 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.      | No IC signal in negative control but positive signal in positive control | Error in reaction set-up   | 1. Prepare a uniform master mix for all tests, distribute it into individual tubes, and then add the sample to each tube.   |
| 4.      | No signal with positive controls   | Incorrect programming of the temperature profile of the thermocycler | Compare the temperature profile to the manual.  |

### Safety Information

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




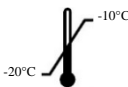




### Disposal

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 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).

### Symbols:

|   |                               |   |                                  |
|---|-------------------------------|---|----------------------------------|
|    | Manufacturer                  |    | Do not use if package is damaged |
|  | Batch code                    |  | Temperature limit                |
|  | Date of manufacture (YYYY-MM) |  | Consult instructions for use     |
|  | Use-by date (YYYY-MM)         |  | Catalogue number                 |

Identification No.: PIMBPCR167

Rev.No.:10

Date of Issue: 2025-06

### Disclaimer :

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