# $Opti^{TM} MP-qPCR$ (Instructions for use)

Real-time PCR for detection of *Mycoplasma* spp. Catalog Number LABS-007

### 01. Product description

The Opti<sup>TM</sup> MP-qPCR is an in vitro diagnostic reagent that tests for the presence of bacteria in samples suspected of *Mycoplama* species infection using real-time PCR.

The qPCR mix includes buffer, enzyme, primers and fluorescence probes for optimized real-time qPCR amplification of the *Mycoplasma* spp. and exogenous internal control. Exogenous internal control is used to monitor for the presence of PCR inhibitors.

#### 02. Contents and storage

No.	Component	Amount	Storage[*]
1	qPCR mix (Taq DNA polymerase Primers & probe)	8 T x 12 Strips (1tube 15μℓ)	-20°C
2	Positive control	100 µL, 1 Vial	

<sup>[\*]</sup> See packaging for expiration date.

#### 03. Set up the PCR reactions

- 1. Blood, serum, and respiratory tissue samples can be used for testing, and DNA should be extracted using fresh samples. For DNA extraction methods, refer to each manufacturer's instructions.
- 2. Add 5  $\mu$ L of template DNA to the 8-strip tube.
- 3. To the positive control reaction tubes, add 5  $\mu$ L of the positive control (total 20ul).
- 4. To the negative control reaction tubes, add 5  $\mu$ L PCRgrade H<sub>2</sub>O (or negative extract).
- 5. Gently mix the reactions without creating bubbles (do not vortex) and then centrifuge briefly to bring the contents to the bottom of the plate wells or tubes.

#### 04. Set up and run the real-time qPCR

- 1. Following the manufacturer's instructions, set up the real-time PCR run using the following parameters.
  - 1) Reaction volume: 20 µL
  - Select detectors and assign Fluorescence probe reporter dyes and quenchers for each tube used in the analysis.

Target	Reporter	Quencher
<i>Mycoplasma</i> spp.	FAM dye	NFQ-MGB
Exogenous IC	HEX dye	NFQ-MGB

**NOTE:** The FAM/HEX should be detected by the same tube.

3) Thermal cycling program:

Step	Temperature	Time	Repetition
1	95°C (Pre-denaturation)	3 min	1
2	95°C (Denaturation)	3 sec	10
	60°C (Annealing)	30 sec	10
3	95°C (Denaturation)	3 sec	40
	55°C (Annealing)	30 sec	( <u>Plate read</u> )

### 05. Guidelines for data analysis

- 1. This diagnostic kit is a nested PCR method that does not measure fluorescence for the initial 10 cycles, unlike general real-time PCR, so the calculated Ct value may appear about 10 cycles less than the actual number of PCR cycles, so care should be taken when comparing the results with other diagnostic reagents.
- 2. The result of the reaction can be confirmed by the Ct value automatically analyzed by the real-time PCR device. If necessary, the baseline threshold can be manually set to 350 for each dye (mostly based on automatic settings).
- 3. The positive control (PC) is judged as normal results when the Ct value is confirmed to be 25 or less (Ct<25).
- 4. Analysis results based on fluorescent dye.

Amplification signals	Result
FAM/HEX positive	Mycoplasma spp. positive
FAM negative HEX positive	Mycoplasma spp. negative
FAM, HEX negative PC positive	If the concentration of sample DNA is too high or too low, Internal control may not be detected. *Retesting is required.

5. Refer to the table below for criteria for reading results according to Ct value.

Acceptability	Ct value	Result
Optimal	$10 \le Ct < 30$	Positive
Acceptable	30≤ Ct <35	Positive Since the concentration of the target gene is low, retest at higher concentration to obtain more stable results.
Invalid	35≤ Ct	Negative

**NOTE:** The range of Ct values with high reliability (over 80% on average) is within 10 to 25. It the Ct value is over 35, the target DNA used in qPCR is a small amount of 1 to 3 strands. So, the error range is large. These results are unreliable.

## 06. Period of use

This product has a shelf life of 12 months from the date of manufacture and 6 months after opening at -20°C.

### 07. Technical support

Use the information below to inquire about product specifications or technical issues.

Phone: +82-43-249-7500/FAX:+82-43-249-7501 Homepage: <u>http://www.optipharm.co.kr</u> Email: pcrts@optipharm.co.kr

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DNA/CFX96/Rev.0

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# DNA/CFX96/Rev.0

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the threshold value to 350.

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