

## Product Information:

**Mycoplasma PCR Detection Kit Catalog Number:**  
**M209001**

**Sizes: 100 tests (1.8mL)**

**Stored at: -20°C**

### Kit contains:

- 1xGreen PCR mixture**, contains 1x PCR buffer, primer sets, Taq DNA polymerase, dNTP (dATP, dCTP, dGTP, dTTP), PCR buffer stabilizer, RNase/DNase inhibitors and inert green tracking dyes (**Blue Cap**). The inert green tracking dye migrates blue and yellow, at approximately 4kb and 50bp that help visualize the electrophoresis progress.
- Positive control (Yellow Cap)**

### Features and benefits:

- 1. Easy:** samples are prepared directly from culturing cell supernatant, frozen cells or cell lysates.
- 2. Sensitive:** able to detect 1fg Mycoplasma DNA, equivalent to one or two genome copies of the 16S rRNA coding region.
- 3. Broadest:** able to detect up to 117 species of Mycoplasma. (**see P.S.\***)

### Protocol:

#### 1. Sample preparation:

**A: 500 ul supernatant from cell culture medium and heat at 95°C for 5 minutes.**

**or B: 100-1000 cells in 100ul PBS from frozen cells and heat at 95°C for 5 minutes. (Note\*)**

**or C: 5-10ul cell Lysates in 100ul PBS and heat at 95°C for 5 minutes.**

#### (Note\*):

**Simply centrifuge cells and re-suspend in 100 ul PBS for removing DMSO in medium. After heated, the samples are vortex for 5 seconds and centrifuged at 13,000 rpm for 2 minutes at room temperature. The supernatant will be used as DNA template in PCR detection.**

**2. Briefly centrifuge the heated samples for 5-10 seconds to pellet cellular debris before adding to the PCR mixture.**

#### 3. Preparation of PCR Master Mix for a single reaction (total volume: 20uL)

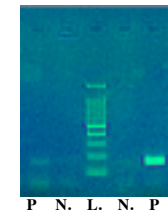
Component	Volume (µL)
1. 1xGreen PCR Mix (Blue cap)	18uL
2. Heated samples from step 1.  or Positive control (yellow cap)	2uL
<b>Total volume per reaction:</b>	<b>20uL</b>

**4. Run reactions in the normal PCR thermal cyclers as following cycling parameters**

Step:	Temperature:	Time:
1). Denaturation (1 cycle)	94°C	2 minutes
2). Cycles (39 cycles):		
Denature	94°C	30 seconds
Annealing	55°C	30 seconds
Extension	72°C	30 seconds
3). Cool down to	4-8°C	End

**5. Run 1.5-2% agarose gel at 120v for 30 minutes and evaluate results with a distinct DNA band. A distinct 167bp or 130bp band indicates positive control or Mycoplasma contamination.**

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L (Fluo-DNA ladders, FD-100)

**P.S\*: Mycoplasma species detected by ZmTech ® Mycoplasma PCR/RT-PCR Detection Kits (Cat. M208001/M209001/M20802)**

1 Acholeplasma entomophilum	25 M. cricetuli	49 A. laidlawii	73 M. buccal	97 Spiroplasma apis
2 Acholeplasma modicum	26 M. cynos	50 M.moatsii	74 M. buteonis	98 M. maculosum
3 Mesoplasma entomophilum	27 M. dispar.	51 M.mobile	75 M. californicum	99 M. spermatophilum
4 Entomoplasma somnilux	28 U. diversum	52 M. molare	76 M. canadense	100 M. spumans
5 Anaerplasma anaerobium	29 A. oculi	53 M. mustelae	77 M. canis	101 M. lipophilum
6 Asteroleplasma abastoclassicum	30 M. ellychniae	54 M. muris	78 Capri	102 M. verecundum
7 Asteroleplasma varium	31 M. equirhinis	55 M. mycoides sub.	79 M. adleri	103 M. yeatsii
8 Capripneumoniae	32 M.equigenitalium	56 M. neurolyticum	80 M. capricolum	104 M. lipofaciens
9 M. agalactiae	33 M.falconis	57 M.opalescens	81 M. caviae	105U. canigenitalium
10 M. agassizii	34 M. fastidiosum	58 M. orale	82 M. arginini	106 Spiroplasma citri
11 M. alkalescens	35 M. faucium	59 M. ovipneumoniae	83 M.citelli	107 M. SP2
12 M.alligatoris	36 M.felifaucium	60 Ovine ureaplasmas	84 M.cloacale	108 M.sualvi
13 M.anseris	37 M. felis	61 M.oxoniensis	85 M.collis	109 Ureaplasma urealyticum
14 M. columbinassale	38 M. fermentans	62 U.parvum	86 M. anatis	110 M. imitans
15 M. cavipharyngis	39 M. flocculare	63 M. penetrans	87 M. columbinum	111 M. SP1
16 M. arthritis,	40 M. gallinarum	64 M. phovirninis	88 M. columborale	112 M. synoviae
17 M. auris	41 M .gallinaceum	65 M. pirum	89 M. conjunctivae	113 M.lagogenitalium
18 M. bovigenitalium	42 M. gallisepticum	66 M. pneumoniae	90 M. corogypsi.	114 M.iguanae
19 M. bovirhinis	43 M.gallopavonis	67 M.primatum	91 M. hominis	115 M. salivarium
20 M. hypopneumoniae	44 M. gateae	68 M. pulmonis,	92 Bovine group 7	116 M. iowae
21 M. bovis	45 M. genitalium	69 M. pullorum	93 M. hyopharyngis	117 M. leopharyngis
22 M. hyorhinis cultivar a	46 M glycopophilum	70 M. putrefaciens	94 M. bovoculi	
23 M. stumi	47 M. indiense	71 M. meleagridis	95 M. hyorhinis	
24 M. subdolum	48 M. ine	72 M.gypis	96 M. hyosynoviae	

**Precautions and Disclaimer:** This product and procedure described are intended for R&D use only. Purchase of this product does not convey a license to perform any patented process.