

Product Information

ZmTech® Mycoplasma qPCR Detection Kit

Catalog Number: **M208002**

Storage Temperature: **-20°C**

Kit contains:

1. Ready-to-use 1xPCR mixtures, including optimization of 1x PCR buffer, primer sets, Taq DNA polymerase dNTP (dATP, dCTP, dGTP, dTTP), PCR buffer stabilizer, RNase/DNase inhibitors and Green Dye (Blue Cap).
2. 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.*)

Storage and stability:

Store at -20°C in a constant-temperature freezer.

I. Protocol:

1. Sample preparation:

- A: 100ul supernatant from cell culture medium and heat at 95°C for 5 minutes.
 or B: 10³-10⁴ cells in 100ul PBS from frozen cells and heat at 95°C for 5 minutes. (Note*)
 or C: 2ul cell Lysates in 100ul PBS and heat at 95°C for 5 minutes.

(Note*):

Simply centrifuge cells and re-suspend in 100ul 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: 25uL) in a 0.2 ml or 0.5ml microtube

Component	Volume (µL)	Final Concentration
1. PCR Mixture (Blue cap)	22.5	(1x)
2. Heated samples	2.5	
Total volume	25.0	

Component	Volume (µL)	Final Concentration
1. PCR Mixture (Blue cap)	22.5	(1x)
2. Positive control	2.5	
Total volume	25.0	

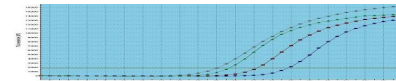
4. Run reactions in the Real Time PCR thermal cyclers as following cycling parameters

Step: Temperature: Time:

1. Denaturation (1 cycle)
94°C 10 minutes (Essential)
2. Cycles (39 cycles):
Denature 94°C 30 seconds
Annealing 55°C 30 seconds
Extension 72°C 30 seconds
3. Melting (Dissociation) Curve Analysis (see instrument manufacturer guidelines)

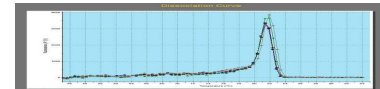
5. Evaluate results:

Figure 1. Evaluate Mycoplasma genome copies in each PCR reaction and determine the density of Contamination.



Delta Rn vs Cycle

Figure 2. Determine Mycoplasma species



Dissociation/melting-curve analysis

II. Technical Tips:

• Approximately evaluate the density of Mycoplasma contaminations from representative RT-PCR amplification curves (Delta Rn vs Cycle)

• Using the dissociation-curve analysis (melting peak value) to determine Mycoplasma species in cell lines:

Species: *M. fermentans* *M. hyorhinis* *M. orale* *M. salivarium* *M. arginini*
 Tm°C: 79.4°C (H) 79.0°C (H) 79.2°C (H) 77.6°C (H) 77.7°C (H)
 Tm°C: 81.0°C (M) 81.0°C (M) 81.5°C (P) 79.5°C (M) 81.5°C (R)

• 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 in the test sample.

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.

P.S*: Mycoplasma species detected by ZmTech® Mycoplasma PCR/RT-PCR Detection Kits (Cat. M208001/M208002)

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

P.S: The bacterial strains are not be detected by ZmTech® Mycoplasma PCR/RT-PCR Detection Kits (Cat. M208001/M208002)**

Actinomyces israelii	Chromatium vinosum	Haemophilus influenzae	Ornithobacterium rhinotracheale	Staphylococcus pneumoniae
Bacillus subtilis	Clostridium innocuum	Haemophilus parainfluenzae	Pasteurella avium	Staphylococcus pyogenes
Bacteroides nodosus	Clostridium ramosum	Helicobacter felis	Pasteurella gallinarum	Streptomyces ambofaciens
Borrelia burgdorferi	Corynebacterium variabilis	Lactobacillus cateniforme	Pasteurella multocida	Streptococcus mutans
Campylobacter hyointestinalis	E. coli	Lactobacillus fermentum	Propionibacterium acnes	Streptococcus pneumoniae
Candida albicans	Escherichia coli	Megasphaera elsdenii	Pseudomonas aeruginosa	Streptococcus pleomorphus
Chlamydia psittaci	Erysipelothrix rhusiopathiae	Mycobacterium tuberculosis	Salmonella Typhimurium	Thermus thermophilus
Chlamydia trachomatis	Eubacterium bifforme	Neisseria gonorrhoeae	Staphylococcus aureus	
Chlamydia pneumoniae	Gardnerella vaginalis	Nocardia asteroides	Staphylococcus epidermidis	