ZmTech Scientifique Innovation and Development of Laboratory Accessories and Bio-Technologies

® •ZmTech Mycoplasma PCR/RT-PCR Detection Kits (Cat. M208001/M208002)

Kit contains: (All-IN-ONE PCR Mastermix)

1. **Ready-to-use SYBR Green PCR Mixtures, including optimization of 1x PCR Buffer, Primer Sets, Taq DNA Polymerase, dNTP (dATP, dCTP, dGTP, dTTP), PCR Buffer Stabilizer, RNase/DNase inhibitors and SYBR Green I dye (Blue Cap)**
2. **Positive control (Yellow Cap)** **Stored at -20°C**

Supplemental Requirements:

1. **PCR/Rea-time PCR devices**
2. **PCR reaction tubes or Glass Capillaries**

Features and Benefits:

1. **Easy: Samples are prepared directly from culture cell supernatant, frozen cells or cell lysates.**
2. **Safest: Samples directly get from frozen cells, not need to re-culture cells in incubator and handle in hood.**
3. **Sensitive: able to detect 1fg Mycoplasma DNA, equivalent to one or two genome copies of the 16S rRNA coding region.**
4. **Broadest: able to detect up to 117 species of Mycoplasma. (see P.S.*)**

Protocol: (Sample can be prepared from culturing cells or frozen cells or cell Lysates)

1. **Sample preparation A: 100ul supernatant from cell culture medium and heat at 95°C for 5 minutes.**

   Sample preparation B: 1-10 x 10^3 cells in 100ul PBS from frozen cells and heat at 95°C for 5 minutes. (Note*)

   Sample preparation C: 2ul cell Lysates in 100ul PBS and heat at 95°C for 5 minutes.

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

2. **Run reactions in the spectrofluorometric thermal cyclers (Suggested Protocols: *SP)**

***SP 1.**
Run reactions in the Normal PCR thermal cyclers as following cycling parameters in a 25ul plastic tube.

Add 2.5ul Samples /2.5ul positive control / 2.5ul water (Negative)
into 22.5ul Ready-to-use Mixtures (Blue cap)
Total: 25ul running reaction into a PCR Tube

	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.	Cool down to	4-8°C	End → Run 2% agarose gel at 120v for 30 minutes

***SP 2.**

Run reactions in the Real-Time PCR thermal cyclers as following cycling parameters in a 25ul plastic tube.

Add 2.5ul Samples /2.5ul positive control / 2.5ul water (Negative)
into 22.5ul Ready-to-use Mixtures (Blue cap)
Total: 25ul running reaction into a PCR Tube

	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)	

***SP 3.**

Run reactions in the Real-Time PCR thermal cyclers as following cycling parameters in a 20ul capillary tube.

Add 2 ul Samples /2ul positive control / 2ul water (Negative)
into 18 ul Ready-to-use Mixtures (Blue cap)
Total: 20ul running reaction into a capillary tube

	Step:	Temperature:	Time:	Temperature Transition Rate
1.	Denaturation (1 cycle)	94°C	10 minutes	20°C/second
2.	Cycles (35 cycles):			
	Denature	94°C	15 seconds (none)	20°C/second
	Annealing	55°C	15 seconds (none)	20°C/second
	Extension	72°C	8 seconds (none)	20°C/second
	Detection	72°C	0 seconds (single)	20°C/second
3.	Melting(1 cycle)	52°C	1 minute (none)	20°C/second
			(95°C, 0 Second, 0.15°C/second, con.)	
4.	Cooling (1cycle)	40°C	30 seconds (none)	20°C/second

(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. This supernatant will be used as DNA template in PCR detection.

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Test Evaluation:

A) **A 167bp or 130bp DNA band was detected if running contaminated samples in 1.5-2% Agarose gel for 30 minutes at 120V.**
B) **Approximately evaluate the density of Mycoplasma contaminations from representative RT-PCR amplification curves (Delta Rn vs Cycle)**
C) **Determine some Mycoplasma species by melting(dissociation) peak Tm°C**

Figure1. Gel Evaluation: a distinct 167bp or 130bp band indicates positive control or Mycoplasma contamination.

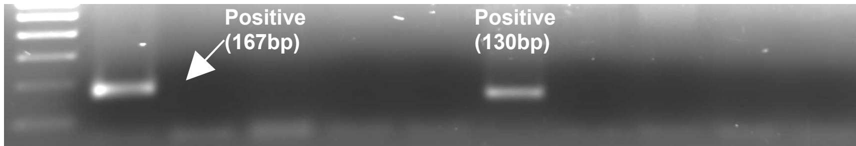


Figure2. Evaluate Mycoplasma genome copies in each PCR reaction and determine the density of Contamination.

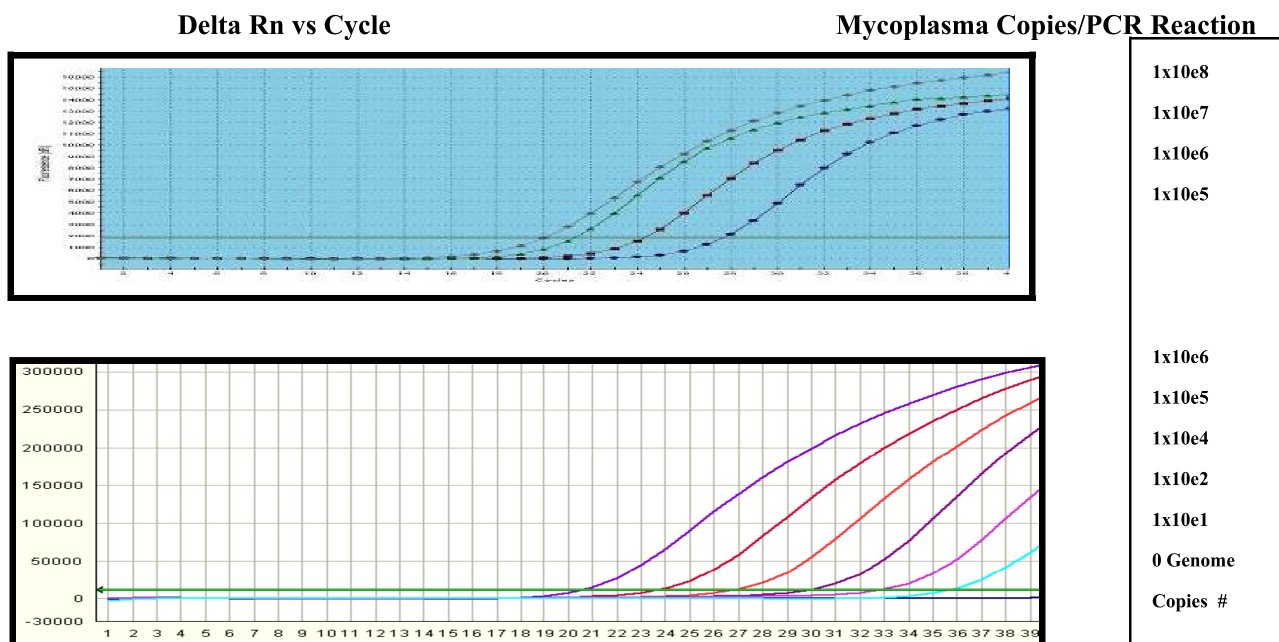
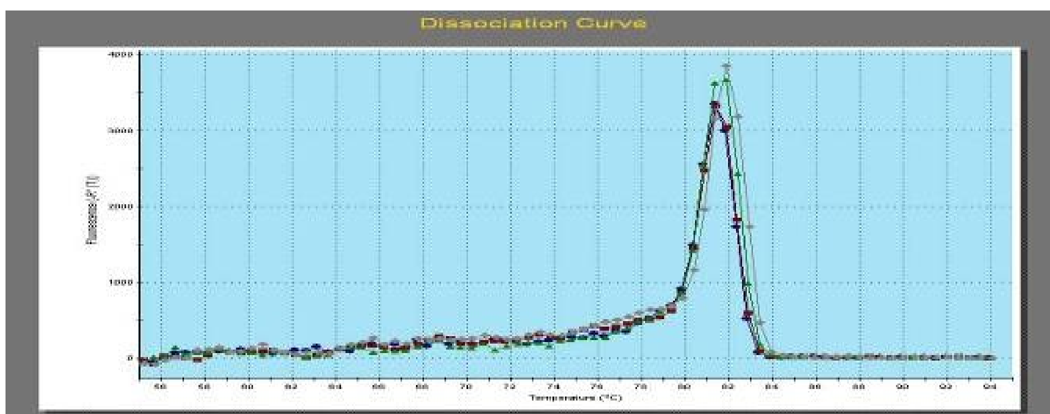


Figure3.Using the dissociation-curve analysis (melting peak value) to determine Mycoplasma species in cell lines:



Species:	*M.* Fermentans	*M.* .hyorhinis	*M.* orale	*M.* Salivarium	*M.* arginini
Tm°C:	79.4°C (H)	79°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)

P.S*: Mycoplasma Species Detected by

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1	**Acholeplasma entomophilum**	41	**M. cricetuli**	81	**Mesoplasma entomophilum**
2	**Acholeplasma modicum**	42	**M. cynos**	82	**M.moatsii**
3	***A. laidlawii***	43	***M. dispar***	83	**M.mobile**
4	**A. oculi**	44	***U. diversum***	84	**M. molare**
5	**Anaerplasma anaerobium**	45	**Entomoplasma somnilux**	85	**M. mustelae**
6	**Asteroleplasma abastoclassicum**	46	**M. ellychniae**	86	**M. muris**
7	**Asteroleplasma varium**	47	***M. equirhinis***	87	**M. mycoides subsp**
8	**M. adleri**	48	***M. equigenitalium***	88	***M. neurolyticum***
9	***M. agalactiae***	49	***M.falconis***	89	***M.opalescens***
10	***M. agassizii***	50	**M. fastidiosum**	90	***M. orale***
11	**M. alkalescens**	51	**M. faucium**	91	***M. ovipneumoniae***
12	**M.alligatoris**	52	**M.felifaucium**	92	**Ovine ureaplasmas**
13	**M.anseris**	53	**M. felis**	93	**M.oxoniensis**
14	***M. anatis***	54	***M. fermentans***	94	**U.parvum**
15	***M. arginini***	55	***M. flocculare***	95	***M. penetrans***
16	***M. arthritidis,***	56	***M.gallinarum***	96	***M.phovirninis***
17	***M. auris***	57	***M.gallinaceum***	97	***M. pirum***
18	**M. bovigenitalium**	58	**M. gallisepticum**	98	***M. pneumoniae***
19	**M. bovirhinis**	59	**M.gallopavonis**	99	***M.primatum***
20	**Bovine Group 7**	60	**M. gateae**	100	***M. pulmonis,***
21	***M. bovis***	61	***M. genitalium***	101	***M. pullorum***
22	**M. bovoculi**	62	***M. glycophilum***	102	**M. putrefaciens**
23	**M. buccale**	63	***M.gypis***	103	***M. salivarium***
24	**M. buteonis**	64	***M. hominis***	104	***M. SP1***
25	**M. californicum**	65	***M. hypopneumoniae***	105	***M. SP2***
26	**M. canadense**	66	**M. hyopharyngis**	106	***M. spermatophilum***
27	**M. canis**	67	**M. hyorhinis cultivar a**	107	***M. spumans***
28	**Capri**	68	**M. hyorhinis**	108	***Spiroplasma apis***
29	**Capripneumoniae**	69	**M. hyosynoviae**	109	**Spiroplasma citri**

30	*M. capricolum)*	70	M.iguanae	110	M. stumi
31	*M. Caviae*	71	M. imitans	111	*M. subdolum*
32	M. cavipharyngis	72	M. indiense	112	*M.sualvi*
33	M.citelli	73	*M. iners*	113	M. synoviae
34	M.cloacale	74	M. iowae	114	Ureaplasma urealyticum
35	M.collis	75	M.lagogenitalium	115	*U. canigenitalium*
36	M. columbinassale	76	*M. leopharyngis*	116	M. verecundum
37	M. columbinum	77	*M. lipofaciens*	117	M. yeatsii
38	M. columborale	78	M. lipophilum		
39	M. conjunctivae	79	M. maculosum		
40	M. corogypsi	80	M. meleagridis		

Other bacterial strains are not be Detected by

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Actinomyces israelii	Chromatium vinosum	Haemophilus influenzae	Ornithobaterium rhinotracheale	Staphylococcus pneumoniae
Bacillus subtilis	Clostridium inocuum	Haemophilus parainfluenze	Pasteurella avium	Staphylococcus pyogenes
Bateroides nodosus	Clostridium ramosum	Helicobacter felis	Pasteurella gallinarum	Streptomyces ambofaciens
Borrelia burgdorferi	Corynebacterium variabilis	Lactobacillus catenaforme	Pasteurella multocida	Streptococcus mutans
Campylobacter hyointestinalis	E. coli	Lactobacillus fermentum	Propionibacterium acnes	Streptococcus pneumoniae
Candida albicans	Escherichia coli	Megasphaere elsdenii	Pseudomonas aeroginosa	Streptococcus pleomorphus
Chlamydia psittaci	Erysipelothrix rhusiopathiae	Mycobacterium tuberculosis	Salmonella Typhimurium	Thermus thermophylus
Chlamydia trachomatis	Eubacterium biforme	Neisseria gonorrhoeae	Staphylococcus aureus	
Chlamydiapneumoniae	Gardnerella vaginalis	Nocardia asteroides	Staphylococcusepidermidis	

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