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Mycoplasma PCR Detection Kit

Cat #: BMC1040 Size: 200 T/1000 T

	Mycoplasma PCR Detection Kit		
REF	**Cat #:** BMC1040	LOT	**Lot #:** Refer to product label
	Applicable samples: Cell culture supernatant, laboratory animal secretions, serum and other biomaterial samples		
	Storage: Stored at -20℃ for 12 months		

Assay Principle

This Mycoplasma PCR Detection Kit utilizes Nested PCR technology to amplify and detect the conserved region specific fragments of Mycoplasma 16S-23S rRNA sequence. It can be directly used for rapid detection of biomaterial samples such as cell culture supernatant, laboratory animal secretions, serum, etc. It can quickly, efficiently, and sensitively detect the presence of Mycoplasma contamination.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	200 T	1000 T	
1st PCR Primer Mix (25×)	200 μL	1 mL	-20℃
2nd PCR Primer Mix (25×)	200 μL	1 mL	-20℃
Positive Control Template	100 μL	500 μL	-20℃

Materials Required but Not Supplied

- PCR tube, precision pipettes, disposable pipette tips
- Centrifuge, PCR amplification instrument, horizontal electrophoresis instrument
- 2×PCR Mix, agarose, DNA Marker
- Sterile water

Assay Procedure

A. 1st PCR reaction

1. Melt and mix the various solutions required for the PCR reaction, and set the PCR reaction on an ice bath according to the following table:

Regents	Test	Negative Control	Positive Control
2×PCR Mix	12.5 μL	12.5 μL	12.5 μL
1st PCR Primer Mix (25×)	1 μL	1 μL	1 μL
Sterile Water	9.5 μL	11.5 μL	9.5 μL
Samples	2 μL	/	/
Positive Control Template	/	/	2 μL
Total Volume	25 μL	25 μL	25 μL

Note: To prevent contamination of the Positive Control Template, it is necessary to add the Positive Control Template to the Positive Control group after adding Samples/Sterile water to the Test group/Negative Control group.

2. Perform PCR reaction according to the following conditions:

94℃	5 min	
94℃	30 s	30-35 cycles
56℃	30 s	
72℃	1 min	
72℃	5 min	
4℃	Forever	

B. 2nd PCR reaction

1．Set the PCR reaction on an ice bath according to the following table:

Regents	Test	Negative Control	Positive Control
2×PCR Mix	12.5 μL	12.5 μL	12.5 μL
2nd PCR Primer Mix (25×)	1 μL	1 μL	1 μL
Sterile Vater	11 μL	11 μL	11 μL
1st PCR Products	0.5 μL	0.5 μL	0.5 μL
Total Volume	25 μL	25 μL	25 μL

2．Perform PCR reaction according to the following conditions:

94℃	5 min	
94℃	30 s	30-35 cycles
56℃	30 s	
72℃	1 min	
72℃	5 min	
4℃	Forever	

3．After the reaction, take 7 μL of each of the 1st and 2nd PCR reaction products for 1-2% agarose gel electrophoresis. If only to determine whether there is mycoplasma contamination, 1-2% agarose gel electrophoresis can be used. If the species of mycoplasma contaminate needs to be roughly inferred from the fragment size of the PCR product, 2% agarose gel electrophoresis is recommended.

Typical Data

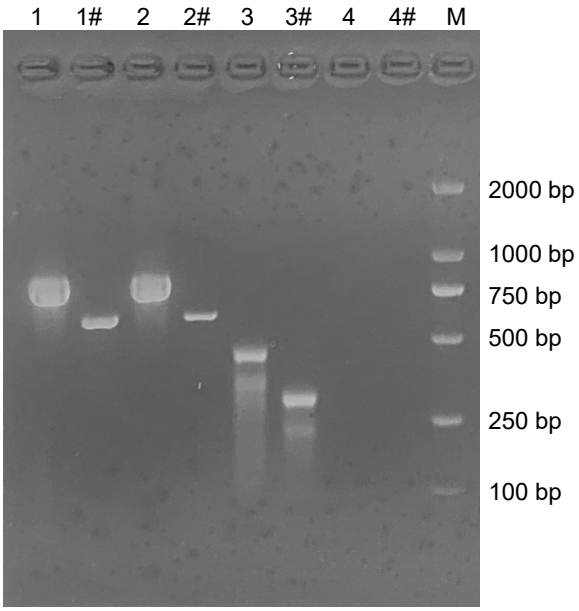

Figure 1. Agarose gel electrophoresis of PCR amplification products using this Mycoplasma PCR Detection Kit
1, 2, 3, and 4 are the products of 1st PCR; 1#, 2#, 3#, 4# are the corresponding products of 2nd PCR. The templates for each lane are: 1 and 1#, Positive Control Template; 2 and 2#, Positive Control Template 1:10 dilution; 3 and 3#, Mycoplasma contaminated cell supernatant; 4 and 4#, sterilized water. M, DNA Marker.

Table 1. Reference table of common mycoplasma species and length of products from 1st PCR and 2nd PCR

Species	1st PCR (bp)	2nd PCR (bp)
Positive Control Template	820	599
Mycoplasma arginini	370	145
Mycoplasma arthritidis	408	157
Mycoplasma capricolum	415	221
Mycoplasma fermentans	492	195
Mycoplasma hominis	370	148
Mycoplasma hyopneumoniae	682	238
Mycoplasma hyorhinis	452	211
Mycoplasma neurolyticum	502	196
Mycoplasma orale	424	179
Mycoplasma pulmonis	477	190
Mycoplasma salivarium	403	151
Ureaplasma urealyticum	482	154

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.