MycoDect™ Mycoplasma Detection Kit Protocol

Cells cultured in the absence of antibiotics for more than three days can enhance the PCR signal. Media sample should be derived from cells that are at least 80% confluent.

Sample Preparation from Cell Lysate

1. Aspirate medium from attached cells, and rinse cells twice with DPBS.
2. Add 0.2 mL of trypsin per well of 24-well plate, and incubate at 37° C for 2-3 minutes.
3. Add 0.5 mL of 10% FBS/DMEM medium, and transfer the cell suspension to a 1.5 mL microcentrifuge tube.
4. Centrifuge this 1.5 mL tube containing the cell suspension at 300 x *g* for 5 minutes at room temperature.
5. Aspirate the supernatant. Wash the cell pellet with 1 mL DPBS.
6. Centrifuge the tube again at 300 x *g* for 5 minutes at room temperature.
7. Aspirate the supernatant, and resuspend the cell pellet in 100 µL lysis buffer.
8. Lyse at room temperature for 10 min.
9. Heat the lysates at 95 °C for 5 min.
10. Centrifuge the lysates at 15,000 x *g* for 5 min and collect supernatant.
11. Take 1-2 µL supernatant as template for PCR reaction.

Sample Preparation from Media

1. Collect 100 µL of cell culture medium to a microcentrifuge tube.
2. Centrifuge at 15,000 x *g* for 5 min.
3. Heat the cell culture medium at 95° C for 5 min.
4. Take 1-2 µL medium as template for PCR reaction.

Polymerase Chain Reaction (PCR)

PCR setup with Taq polymerase is shown below. A final volume of 20 µL is recommended for each reaction.

	Test Sample / µL	Positive Control Reaction / µL	Negative Control Reaction / µL
MycoDect™ Primer Mix	15	15	15
MycoDect™ PCR Mix	4	4	4
Sample	1	-	-
Positive Control	-	1	-
Negative Control	-	-	1
In Total	20	20	20

PCR Program

Step	Temp	Time
Initial Denaturation	95°C	4 min
30 Cycles	95°C	15 s
	56°C	30 s
	68°C	30 s
Final Extension	68°C	5 min
Hold	4°C	

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PCR Product Detection

For optimal separation between the mycoplasma band and the control, we recommend 2% agarose gel for electrophoresis.

1. Mix the final products of each PCR reaction with gel electrophoresis loading buffer.
2. Load each sample into individual wells of the agarose gel, including positive control, negative control, and DNA ladder.
3. Electrophorese at the conditions recommended by the gel box manufacturer.
4. Visualize the bands with ethidium bromide.

Limitations

- Use ribonuclease-free (RNase-free) reagents and supplies when running this assay.
- Do not mix or substitute reagents with those from other sources or lots.
- The kit should not be used beyond the expiration date on the kit label.
- Any variation in diluents, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can alter assay performance.
- This assay detects the following species: *M. hyorhinis*, *M. arginini*, *M. fermentans*, *M. orale*, *M. pirum*, *M. hominis*, *M. salivarium*, and *A. laidlawii*.
- A negative result does not indicate that other species of mycoplasma are absent. Additionally, mycoplasma may be present at levels below the detectable limits of this kit.
- Cell cultures that are visibly contaminated (i.e., turbidity and yellow media) are probably due to *E. coli* or fungal infection and should not be tested. Visibly contaminated cell cultures should be discarded and fresh cultures should be started from frozen stock.
- This assay cannot be used for mycoplasma species identification.

Explanation of Results

To determine whether the sample is contaminated with mycoplasma, ensure that both positive control and negative control give expected results.

PCR template	PCR product(s)	Interpretation
Positive control	270 and 150 bp bands	Expected control result
	No band	Failed PCR reaction
Negative control	One 150 bp or no band	Expected result
	Two bands	Contaminated reagents
	No band	Failed PCR Reaction
Sample	270 and 150 bp bands	Mycoplasma contamination
	270 bp band only	Severe mycoplasma contamination
	150 bp band only	No mycoplasma contamination
	No band	No mycoplasma contamination