MycoDect™ Mycoplasma Detection Kit

Catalogue number: MD050; Size: 50 reactions; Prices: $209

Catalogue number: MD200; Size: 200 reactions; Prices: $450

Product Description

Mycoplasmas are small, round or filamentous prokaryotic organisms that lack cell walls. It has been estimated that at least 15%-35% cell cultures are contaminated with mycoplasma. Mycoplasma contamination can modify many aspects of cell physiology without causing obvious medium cloudiness and apparent effect on cell growth. Mycoplasma contamination affects up to 80% of continuous cell cultures. If undetected, mycoplasma contamination can have significant effects on the quality and reliability of your cell culture preparations. Due to their small size and deformability, mycoplasmas can pass through 0.22μm filter. The lack of cell wall makes mycoplasma unresponsive to common antibiotics that target cell wall synthesis. It is essential that all cell stocks and new cultures entering a facility are tested for the presence of mycoplasma and all cell cultures should be tested routinely (e.g. once every 2-3 months) in order to maintain a mycoplasma-free environment.

Many methods are available for detection of mycoplasma, including isolation in broth/agar culture, direct or indirect fluorescence staining, ELISA, immunostaining, direct or indirect PCR. Among those methods, direct PCR is the most sensitive, specific and convenient method when the primer design is optimized.

The MycoDect™ Mycoplasma Detection Kit is designed to detect mycoplasma infection in cell cultures in less than two hours. It can detect mycoplasma from both cell lysates and cell culture media. The sensitivity is up to 10-20 copies of target DNA, which translates to less than 10 mycoplasma species. This kit detects the top five common species of mycoplasma, *M. Hyorhinis*, *M. Arginini*, *M. Orale*, *M. Fermentans*, *A. laidlawii*, which represent 98% of tissue culture infections. It can also detect Mycoplasma, Acholeplasma, Ureaplasma and Spiroplasma from other genera.

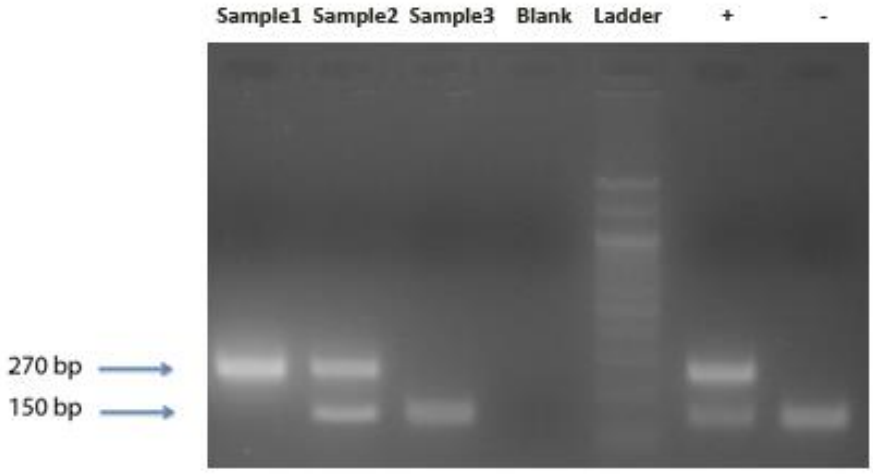
Highlights:

- Sensitive and easy to use
- Detect Mycoplasma contamination in less than two hours.
- Detects the most common contamination species, including *M. hyorhinis, M. arginini, M. orale, M. fermentans*, and *A. laidlawii*, which represent 98% of tissue culture infections.
- Detects genera such as Acholeplasma, Ureaplasma, and Spiroplasma.

Kit Components

- MycoDect™ PCR Mix
- MycoDect™ Primer Mix
- Positive Control
- Negative Control
- Lysis Buffer

Example of Experimental Results



Sample 1 shows severe mycoplasma contamination. Sample 2 gives positive results for mycoplasma with both 270 and 150 bp bands. Sample 3 gives negative results with 150 bp band only.

Product Specifications

Product Name	MycoDect™ Mycoplasma Detection Kit
Catalog #	MD050, MD200
Size	50 reactions (MD050) / 200 reactions (MD200)
Shipping	Dry ice or blue ice
Storage and Stability	Store at -20° C. This product is stable for 12 months when stored as directed.
Quality Control	Each lot of MycoDect™ Mycoplasma Detection Kit is functionally tested.
Restricted Use	For Research Use Only. Not for use in diagnostic or therapeutic procedures.

Documents:

Protocol

Product Specification Sheet

Certificate of Analysis

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	

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