

# PCR Mycoplasma Detection Kit

CAT No. APL-5003-1

## DESCRIPTION

PCR Mycoplasma Detection Kit is designed for the identification of mycoplasma-contaminated cell culture. Mycoplasma infection can alter the infected cell at molecular level, cell growth rate, and morphology. Contamination directly affect the accuracy and reproducibility of experiments. This assay kit allows detection of various mycoplasmas species with high degree of sensitivity.

## CONTENT

	<b>APL-5003-1</b> 20 tests (25 µL/test)
Taq DNA polymerase	25 µL
2X Reaction Buffer	270 µL
Mycoplasma Universal Primer	45 µL
Mycoplasma Positive Control	45 µL
Mycoplasma Free Water	200 µL

## STORAGE

This product should be stored at -20°C and avoid repeated freeze-thaw cycles to retain the maximum activity. When in use, always keep the reaction mix on ice.

## PROTOCOL

### Sample preparation

- The contaminated cell should remain in culture for at least 24~72 hours.
- Collect 1 mL target culture medium into a 1.5 mL micro-centrifuge tube and centrifuge it for 5 mins at 2000 x g to pellet cells and debris. Use the supernatant as the test sample. The test sample can be stored at -20°C for later use.
- Setting the reactions according to the following table:

Component	Sample	Positive Control	Negative Control
Taq DNA polymerase	1 µL	1 µL	1 µL
2X Reaction Buffer	12.5 µL	12.5 µL	12.5 µL
Mycoplasma Universal Primer	2 µL	2 µL	2 µL
Sample	2 µL	0 µL	0 µL
Mycoplasma Positive Control	0 µL	2 µL	0 µL
Mycoplasma Free Water	7.5 µL	7.5 µL	9.5 µL
Final Volume	25 µL	25 µL	25 µL

4. Set the parameters as following table and perform PCR:

Steps	Temperature	Time	Cycles
Pre-denaturation	95°C	5 mins	1
Denaturation	95°C	30 secs	30~40
Annealing	55°C	30 secs	
Extension	72°C	60 secs	
Final Extension	72°C	10 mins	1
Holding	4°C	-	-

5. Analysis of amplified products by gel electrophoresis, and the presence of PCR products at 450~550 bp in length (multiple bands is possible) indicates that the tested cell culture is contaminated with mycoplasmas.

### RECOMMENDATIONS FOR OPTIMAL RESULTS

1. Reagents of the mycoplasma detection kit are mycoplasma free. Please do not utilize any other PCR reagents to perform the PCR.
2. Make aliquots of the reagents to avoid contamination and repeated freeze-thaw cycles.

### PRODUCT USE LIMITATION

Research use only.