

Introduction:

Mycoplasma is a prokaryotic organism that is a frequent and occult contaminant of cell cultures. Mycoplasma infection extensively affects cell physiology and metabolism. Animal sera in cell cultures were the main source of Mycoplasma arginini, Mycoplasma hyorhinis or Acholaepasma laidlawii. It is essential that all cell cultures are required to be tested for the presence of Mycoplasma. We have developed a reliable and sensitive PCR-based assay to detect mycoplasma contaminants in cell culture. The kits can detect directly the samples from cell culture lysates, culture medium or serum. The reagents required for PCR reaction are provided and have been optimized for PCR amplification. The PCR reaction employed the universal primers against the 16S rRNA conserved genome region of the most mycoplasma strains. The kits include the sufficient volume of components for 50 (CB6969-100) assays.

Mycoplasma universal detection Kit (CB6969-100) for 100 assays

Component	Volume	Storage
Cell lysis buffer	10 ml	4 °c
PCR master mix (2x)	1.5 ml	-20°c
Universal Primers (100 µM)	250 µl	-20°c
Sterilized PCR-grade water	1 ml	-20°c

DNA sample preparation from cell cultures for mycoplasma detection protocol:

1. Cell Harvest:

A. Suspension cells: Count cells. 10⁴ - 10⁵ cells are needed for the assay.

*Use of more than 10⁶ cells per sample may result in false-positive results.

B. Adherent cells: Scrape the cells into the existing culture media and suspend.

*Do not treat cells with trypsin or EDTA as these agents disrupt mycoplasma.

2. Transfer 1 mL cell suspension into the Sample Lysis Tubes and centrifuge at 10,000 rpm for 5 minutes at 4°C.

3. Carefully remove and discard the supernatant.

4. Add 1 µl Proteinase K and 3 µl RNase A to the resuspended pellet.

*Store RNase A and Proteinase K at -20°C.

5. Resuspend the cell pellet with 96 µL Lysis Buffer by vortexing.

6. Incubate the resuspended cell pellet at 56°C for 5 minutes using a heating block to lyse the cells and degrade the proteins.

7. Heat the samples at 95°C for 10 minutes to inactivate the protease.

8. Spin down cell debris at 13,000 rpm for 5 minutes at 4°C. Transfer supernatant to a new microcentrifuge tube. Samples are now ready for PCR.

Note: Skip step 1 to 8, the medium (1~2 µl) from cell cultures can be directly detected by our mycoplasma detection kit.

PCR primers for mycoplasma detection protocol:

The primers used are derived from a conserved region within the 16S rRNA gene and do not detect eukaryotic DNA or bacterial genera with close phylogenetic relation to mycoplasmas such as Clostridium, Lactobacillus and Streptococcus.

PCR reaction assembling Procedure:

PCR master mix (2x) 12.5 μ l
PCR primers (10 μ M): 2 μ l
DNA sample: 2.5 μ l
Add PCR grade water: 8 μ l
Total reaction: 25 μ l

PCR amplification Procedure for Primers:

PCR reaction program is following as:
Step 1: 95°C, 5 min
Step 2: 95°C, 30 Seconds
Step 3: 54°C, 30 Seconds
Step 4: 72°C, 30 seconds
Step 5: step 2-4, 35 cycles
Step 6: 72°C, 10 min
Step 7: 4°C forever

DNA gel electrophoresis protocol:

1. Prepare 1.4% agarose gel.
2. Prepare samples: Add 5 μ L of the PCR product to 3 μ L loading buffer (6x) and 10 μ L H₂O.
3. Mix thoroughly. Load samples and a DNA marker (e.g., 100 bp ladder) onto the gel. Electrophorese until the tracking dye migrates 80% the length of the gel.
4. Results detection: A test sample that is positive for the presence of mycoplasma shows a distinct band at 517 bp. There should be no visible band in the negative control lane.

Product category: Reagents

Applications: to detect mycoplasma contaminants in cell culture, cell culture lysates, culture medium or serum.

Quality control: Cell growth and viability

Shipping information: Dry Ice

Size: 100 assays

Storage conditions: -20°C or colder