For Research Use Only *Not for Diagnostic Use*

Mycoplasma PCR Detection Kit

Cat. No. CSD100 - 100 tests
Storage Temperature -20°c

MycoXpress Mycoplasma Detection kit utilizes the polymerase chain reaction (PCR), which is the method of choice for highest sensitivity in the detection of *Mycoplasma* contamination in cell cultures and other cell culture derived biologicals. The primer set is specific to the highly conserved the 16S rRNA coding region in the mycoplasma genome. This allows for detection of *M. orale, M. hyorhinis, M. arginini, M. fermentans, Acholeplasma laidlawii, M. hominis,* usually encountered as contaminants in cell cultures. Futhermore, this kit can detect *M. pneumoniae, M. salivarium, M. synoviae* and *Ureaplasma* species. Eukaryotic and bacterial DNA is not amplified by *MycoXpress* Mycoplasma Detection kit.

1. Characteristics

- Detect 50 species of mycoplasma.
- Provide validity of test results by internal control.
- Ready-to-use, optimized PCR premix type.

2. Kit Contents

- Primer mix with internal DNA, 200µl (red cap)
- 2xPCR Premix with loading dye, 1ml (blue cap)
- Mycoplasma control DNA, 100µl (yellow cap)
- DNase Free Water, 600µl (white cap)

3. Storage/Stability Conditions

The kit is shipped on cool packs. Upon receipt, store at -20℃. The kit is stable until the expiration date stated on the label.

4. Mycoplasma Detection Protocols

I. Preparation of Sample (Template)

1) Thaw the kit components at room temperature. Vortex and then spin them briefly in a microcentrifuge to collect the material in the bottom of the tube.
2) Transfer 1.5ml of cell culture supernatant to a microcentrifuge tube.
3) Spin at 1,500rpm for 5minutes to pellet cellular debris.
4) Transfer 1ml of supernatant to a fresh tube.
5) Centrifuge at 12,000rpm for 10 minutes to pellet mycoplasma..
6) Decant the supernatant and resuspend the mycoplasma pellet in 100 µl of sterile water.
7) Heat the samples at 98□ for 10min, and vortex for 5~10 sec. Then, centrifuge for 5 min at 12,000 rpm with a microcentrifuge. (*Caution!! Be careful when you heat the sample at 98□ . Heating it in PCR machine with heating cover is recommended.*)
8) Transfer an aliquot of the heated supernatant to a fresh tube. This supernatant will be used as the template in the PCR.

II. PCR reaction

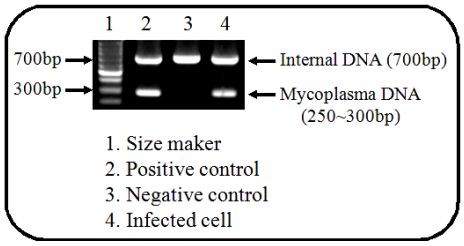
9) Total volume per reaction is 20ul. Add 3~5ul of the heated supernatant as template to a fresh clean PCR tube for PCR.
10) Add 10ul of 2xPCR premix and 2ul of Primer mix to the PCR tube. Adjust the volume of the reaction to 20ul adding DNase free water.
11) Perform PCR reaction as in the following:

Temperature	Time	Cycle
95°C	5 min	1 cycle
94°C	30 sec	35 cycles
55°C	30 sec	
72°C	30 sec	

12) Apply 5~10 µl each of PCR products to the gel electrophoresis.

III. Result

13) When mycoplasma contaminate, a band around 250-300bp is appeared. An internal DNA band around 700bp means a right performance of PCR reaction.



Note:

1) Recommend to perform one negative control without sample and one positive control reaction by adding 1µl of mycoplasma control DNA.
2) If the PCR reaction is inhibited by high FBS concentration, the use of genomic DNA as a template may be helpful.
3) PCR inhibiting substances may accumulate in the medium of hybridoma cell. For this case, the use of diluted sample as a template may be helpful.

5. Related Products

MycoXpress Mycoplasma Elimination Kit
Cat. No. CSE01
Cat. No. CSE03
Cat. No. CSE05

MycoXpress Mycoplasma Prevention Spray
Cat. No. CSP1000 Spray bottle (1 L)
Cat. No. CSP5000 Spray bottles (5 L)

MycoXpress Mycoplasma Free Maintain Reagent
Cat. No. CSF100

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