Mycoplasma Detection Set

Catalog No.: ***COK001***
Pack Size: ***50 standard tests***
Storage: ***-20ºC***

DESCRIPTION

PCR-based detection of mycoplasma contamination of cell culture.

Mycoplasma is a common and serious contaminant of cell cultures. It has been shown that more than 30% of cell cultures in the laboratory are infected with Mycoplasma. In continuous cell cultures, contaminating Mycoplasma may grow slowly without killing the cells but affecting various parameters including altered cellular proliferation and viability, morphological changes, cell transformation, mimicking virus infection, and inresponsiveness to drug treatment, etc., and ultimately leading to unreliable results. Mycoplasma detection is an important and necessary quality control measure.

Many of the testing procedures have been developed, which include DNA staining, enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR), and PCR-based ELISA. M&C Gene Technology provide our research community with reliable reagents and simple protocol, which allow for rapid and highly reproducible detection of mycoplasma contamination.

The primers used in this kit anneal to conserved regions of the Mycoplasma genome, allowing detection of the most common species of Mycoplasma (including M. opalescens, M. arginini; M. fermentans; M. caviae, M. hyorhinis, M. indiense, M. orale, Acholeplasma laidlawii and many more – see table 2 below).

IMPORTANT FEATURES

- ***High Sensitivity***: Sensitive enough to detect trace amount mycoplasma contamination in cell culture medium.
- ***Simplicity***: Only cell culture medium required and no DNA preparation and cell collection.
- ***Broad Detection Range***: Detects common strains of Mycoplasma with a simple protocol.
- ***Species Determination***: The species of mycoplasma can be determined by sequencing the amplified products.

Kit Components	***Volume (μL)***
Mycoplex I (2X)	500
Mycoplex II (2X)	500
MacTaq	50
Water (nuclease-free)	1000
Positive control	50

PROCEDURES

Preparation of template:

Culture cells for at least 3 days to reach >50% confluency. 1-2 μl cell culture medium or positive control will be used as PCR template in a standard 20 μl PCR reaction.

PCR Reaction:

Set up PCR reactions by following the schemes in Table 1. For heavy contamination, only the first round PCR reaction is required; for slight contamination, the second round PCR reaction will give more sensitive measure by amplifying the PCR product from the first round PCR reaction.

Electrophoresis:

Take 10 μL of PCR products to directly load on 1.5%-2% agarose gel. No loading buffer with DNA dye is required.

Table 1: PCR Schemes

1st Round PCR	
Mycoplex I (2X)	10 μL
MacTaq	0.5 μL
Template (culture medium or control)	1 μL
Water (nuclease-free)	8.5 μL

2nd Round PCR	
Mycoplex II (2X)	10 μL
MacTaq	0.5 μL
Template (1st Round PCR product)	1 μL
Water (nuclease-free)	8.5 μL

PCR program

Step 1: 95°C 1'
Step 2: 94°C 30"
Step 3: 55°C 30"
Step 4: 72°C 45"
Repeat step 2-4 for 36 cycles
Step 5: 72°C 10'
Step 6: hold at 4°C

Table 2. Size of PCR product amplified from most common mycoplasma species (in bp)

Species		**1st***	**2nd**
M.hyopneumoniae	肺炎支原体	681	237
M.neurolyticum	溶神经支原体	501	196
M.fermentans	发酵支原体	491	195
M.pulmonis	肺支原体	477	189
M.hyorhinis	猪鼻支原体	448	211
M.orale	口腔支原体	423	179
M.capricolum	山羊支原体	415	179
M.arthritidis	关节炎支原体	408	157
M.salivarium	唾液支原体	403	151
M.hominis	人型支原体	370	148
M.arginini	精氨酸支原体	369	145
M.urealyticum	解脲支原体	482	154

*The first round (1st) PCR band may not show up on agarose gel when using culture media instead of genomic DNA as template, especially when mycoplasma contamination is slight.