

Mycoplasma Detection Set

Catalogue #: COK001/COK001.1

Storage: -20°C

Size: 50/10 reactions

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 irresponsiveness 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. indiensis*, *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.

Procedures:

Preparation of template:

Culture cells for at least 3 days to reach >50% confluency. 2ul cell culture medium will be used as PCR template in a standard 20ul 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 run 1.5% agarose gel.

Kit Components:	COK001	COK001.1
Mycoplex I (2X)	500 µL	100 µL
Mycoplex II (2X)	500 µL	100 µL
MacTaq	50 µL	20 µL
Water (nuclease-free)	1000 µL	200 µL
Positive control	50 µL	20 µL

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Table 1: PCR Schemes

1 st Round PCR	Volume	PCR program
Mycoplex I (2X)	10 µL	
MacTaq	0.5 µL	Step 1: 94°C 30"
Template (culture medium or 2 µL positive control)	2 µL	Step 2: 94°C 30"
Water (nuclease-free)	7.5 µL	Step 3: 55°C 45"
		Step 4: 72°C 45"
2 nd Round PCR		
Mycoplex II (2X)	10 µL	Repeat step 2-4 for 35 cycles
MacTaq	0.5 µL	
Template (1 st Round PCR product)	0.5 µL	Step 5: 72°C 1'
Water (nuclease-free)	9 µL	

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

Species		1 st PCR*	2 nd PCR	Notes
M.hypopneumoniae	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	* 1 st round band sometimes does not show up on agarose gel when using culture media instead of genomic DNA as template, especially when mycoplasma contamination is slight.
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.arthritis	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	

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