

MycoEz

(mycoplasma detection agent)

Catalogue Number: CO002 Storage: -20°C Size: 50 tests

Description:

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. MACGENE Biotechnology provides our research community with reliable reagents and simple protocol, which allows for rapid and highly reproducible detection of mycoplasma contamination. MycoEz offers highly specific and sensitive detection of >200 strains of mycoplasmas in less than 2 hours. This 1X PCR master mix is designed to minimize false positives, ensure quick and reliable routine screening of cell cultures. The primers used in this kit anneal to conserved regions of the mycoplasma genome, allowing detection of all mycoplasma species found in cell culture.

MycoEz includes an internal amplification control, a plasmid containing a non-mycoplasma-specific DNA sequence to test for false negatives. This internal control is simultaneously amplified in the tube with cell culture samples, and should be used with all PCR samples, including the negative and positive controls. This unique design rules out systemic inhibitions, which are often seen among other similar assays that lead to false negatives.

Important Features:

• *High Sensitivity:* Sensitive enough to detect trace amount mycoplasma contamination in cell culture medium.

- *High Specificity:* Uniquely designed primers and PCR buffer condition to minimize false positives.
- Simplicity: Only cell culture medium required and no DNA preparation and cell collection. A complete PCR reaction mix that contains primers, Taq polymerase, and dNTPs is included. Our proprietary MasterMix formulation also contains a gel loading dye, making it convenient for direct gel loading and electrophoresis.
- Broad Detection Range: Detects over 200 Mycoplasma species with a simple protocol.
- *Rapid:* Follow a simple PCR protocol obtaining results in less than 2 hours with as little as 1 minute preparation.
- *Clear results*: Internal and positive controls are included to ensure efficient and accurate PCR interpretation.

Procedures:

<u>Preparation of DNA template (cell culture medium)</u>: Culture cells for at least 3 days to reach >50% confluency. 1 μ l cell culture medium will be used as PCR template in a standard 20 μ l PCR reaction.

<u>PCR Reaction</u>: Set up PCR reactions by following the schemes:

PCR reaction	Volume
MycoEz (1X)	19 µL
Template (culture medium)	1 µL
Total volume	20 µL

PCR program

Step 1: 95°C	1'
Step 2: 94°C	15"
Step 3: 60°C	15"
Step 4: 72°C	30"
Repeat step 2-4	for 33 cycles
Step 5: 72°C	3′

Step 6: hold at 4°C

Electrophoresis:

Take 10 μL of PCRDNA
marproducts to run 1.5%-Mar2% agarose gel. PCR(b.p.
500
from mycoplasma500
300
contaminated samplevields a positive band at100445bp. Internal positive
control yields a 170bp PCR band.



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