NutriCulture Mycoplasma Detection Kit

50 tests

Cat No: MDK50

Shipping : Ship with blue ice.
Storage : Store at -20°C.

General Information

Contamination of cell cultures with bacteria, fungi, and yeasts represents a major problem in cell culture. Although these microorganisms can be easily detected during routine cell culture applications via changes in the turbidity of the culture and visualization under the inverted microscope, a class of bacteria regularly escapes detection. These bacteria belonging to the class of Mollicutes, are commonly known as mycoplasma. Mycoplasma may survive undetected in cell cultures for a long time without any visible effect on the culture.

NutriCulture Mycoplasma Detection Kit offers a sensitive and specific option for the direct detection of mycoplasma in cell cultures via utilization of polymerase chain reaction (PCR). *NutriCulture* Mycoplasma Detection Kit is useful for the routine screening of cell lines newly introduced into the laboratory, for initial analysis of primary cell cultures, and for the periodical monitoring of routinely used cell cultures. The advantages of the *NutriCulture* Mycoplasma Detection Kit are its sensitivity, specificity, speed, cost efficiency, and the potential to screen a large number of samples.

NutriCulture Mycoplasma Detection Kit requires extraction and purification of DNA from the cultured cells to exclude inhibitors of the Taq polymerase that may be present in crude cell culture solutions (which sometimes cannot be eliminated by serial dilutions). *NutriCulture* Mycoplasma Detection Kit includes all essential control reactions like internal, positive, and negative controls.

Protocol

1. Harvest 10^5 – $5x10^5$ suspension or adherent cells. For adherent cells, it is important to not trypsinize cells prior to mycoplasma detection test since trypsin or EDTA may disrupt mycoplasma. Harvest the adherent cells into the existing culture media with a cell scraper.

2. Remove culture media by centrifugation and use cell pellet for genomic DNA isolation using a commercially available kit like EcoPURE Genomic DNA Kit (Cat No: E1075).

3. Use 50 -100 ng DNA for each reaction to prepare the following PCR reaction mixes.

PCR Setup

Component	Test Sample	Positive Control	Negative Control
EcoTaq 2x PCR Master Mix	10 µl	10 µl	10 µl
Myco Primer Mix	5 µl	5 µl	5 µl
Internal Control Primer Mix	2 µl	-	-
Positive Control	-	1 µl	-
Template DNA	50 - 100 ng	-	-
ddH_2O	up to 20 µl	4 µl	5 µl
Total	20 µl	20 µl	20 µl

For further information;
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PCR Reaction Condition

Temperature	Time	Cycles
98°C	30 sec	
94°C	10 sec	
60°C	15 sec	35
72°C	10 sec	
72°C	1 min	
4°C	∞	

4. Prepare a 2% agarose gel with SAFE DNA Gel Stain Solution (20000x) (Cat No: SDGS1).

5. Load 10 µl from each sample and controls along with a DNA Marker 100bp-Green (Cat No: DM100) onto the gel.

6. Electrophorese until the tracking dye migrates 60-70% the length of the gel.

7. Visualize the gel under UV light. A test sample that is positive for the presence of mycoplasma shows a distinct band at around 510 bp with a 105 bp band as internal control indicative of successful run of the PCR reaction. A test sample that is negative for the presence of mycoplasma shows no band at around 510 bp and shows a 105 bp band as internal control indicative of successful run of the PCR reaction. There should be no visible band in the negative control lane.