TransDetect® PCR Mycoplasma Detection Kit

Please read the manual carefully before use

Cat. No. FM311

Version No. Version 2.0

Storage at -18°C or below for two years

Description

TransDetect® PCR Mycoplasma Detection Kit is designed to detect the presence of mycoplasma contamination by PCR in biological materials such as cultured cells. Highly specific primers have been designed to amplify a fragment of 16S rRNA coding DNA that is conserved across all commonly-known mycoplasma species. Using this kit, cell culture supernatants can be tested directly without DNA extraction. The kit features simple operation, fast, high specificity, and high sensitivity. It can detect a total of 54 mycoplasma species, including *M.orale*, *M.pneumoniae*, *M.hyorhinis*, *M.arginini*, *M.fermentans*, *M.salivarium*, *M.pirum*, *M. hominis*, *A.laidlawii*.

Features

• High specificity: Only detect mycoplasma DNA, not eukaryotic and bacterial DNA.

• Simple and easy to use: Requires no genomic DNA extraction, suitable for high-throughput testing of cell samples.

• Positive and negative control: Ensure reliability and accuracy of the results.

Kit Contents

Component	FM311-01 (100 rxns)
TransDetect® PCR Mycoplasma SuperMix II (2×)	2×750 μl
Myco Primer Mix II	200 μl
Myco Positive Control Template III	100 μl
MycoFree Water	1ml

Procedures

1. Sample types

(1) Cell culture supernatant collected after 2–3 days of culture, animal serum, cell culture media, etc.

(2) For cell suspension samples, it is recommended to centrifuge the suspension and use the supernatant for testing.

2. PCR system

To prevent exogenous mycoplasma contamination, wear clean mask and gloves, and set up the following PCR reaction at a designated PCR area. Each experiment should include both negative control (MycoFree Water as template) and positive control ((Myco Positive Control Template III as template).

PCR reaction system (30 μl total volume)

Component	Volume
TransDetect® PCR Mycoplasma SuperMix II (2×)	15 μl
Myco Primer Mix II	2 μl
MycoFree Water	8 μl
Template	5 μl
Total Volume	30 μl

3. PCR program

Temperature	Time	Cycles
95°C	4 min	1
95°C	10 s	35
62°C	25 s	
72°C	10 s	
72°C	5 min	1

4. Agarose gel electrophoresis

Load 10 μl of the PCR product on 1.5% agarose gel for electrophoresis to analyze the amplification results.

5. Interpretation of the result

Analyze and confirm the results of mycoplasma contamination by comparing with the positive and negative control. The size of positive band is about 254 bp.

Note: If bands are observed in the negative control, this may indicate system contamination. It is recommended to repeat the test to confirm the results.

Notes

- Thaw and mix reagents thoroughly before use.
- Antibiotics such as penicillin, streptomycin or serum in the cell culture samples will not affect the detection results of this kit.
- In order to obtain the best detection result, adherent cells reach 80% confluency or suspension cells reach a density of 10^6 cells/ml.
- Throughout the process, please strictly follow the PCR operating standards in a designated area to avoid cross contamination.
- When setting PCR reactions, please wear mask, since oral cavity contains mycoplasma, which may contaminate the sample and cause false positive.
- In order to ensure the reliability and accuracy of the results and meet the publishing requirements of major journals, we recommend that cell cultures and cell culture reagents be regularly tested for mycoplasma contamination. Cell culture is very easy to be contaminated by mycoplasma, so it is recommended to use the *TransSafe*™ Mycoplasma Prevention Reagent (Cat. No. FM501) which has much better effects against mycoplasma to replace the Penicillin & Streptomycin. If Mycoplasma contaminated cells are precious or difficult to culture, it is recommended to use the *TransSafe*™ Mycoplasma Elimination Reagent (Cat. No. FM401, FM411) to eliminate the mycoplasma and rescue the cells.

For research use only, not for clinical diagnosis.

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