# PCR Mycoplasma Test Kit

*Ready-to-use PCR Mix for the detection of Mycoplasma in Cell Culture*

**Product-No. A3744**

## Introduction

The PCR Mycoplasma Test Kit is designed to detect the presence of mycoplasma, since it is common contaminant of biological materials including cultured cells. There are two standard types of testing for mycoplasma contamination: the direct culture method and nucleic acid testing (NAT). Mycoplasma detection by the direct culture procedure is time-consuming and some mycoplasma species are difficult to cultivate. With the NAT method, polymerase chain reaction (PCR) is used to amplify mycoplasma specific DNA, and the results are obtained within a few hours. Using the PCR Mycoplasma Test Kit means that there is no need to prepare PCR primers, label probes with radioisotopes, or to determine polymerase, dNTP's or buffer concentrations. Instead, a ready-to-use, optimized PCR master mix (Reaction Mix) is supplied. Using the Reaction Mix allows the direct loading of PCR products onto agarose gel. The primer set allows detection of various mycoplasma species (e.g., M. fermentans, M. hyorhinis, M. arginini, M. orale, M. salivarium, M. hominis, M. pulmonis, M. arthritidis, M. bovis, M. pneumoniae, M. pirum and M. capricolum), as well as Acholeplasma and Spiroplasma species, with high sensitivity and specificity. The kit contains positive control (DNA template) and internal control (DNA template) to exclude the possibility for PCR inhibition by the test sample (false negative).

| **Product No.** | **A3744,0020** |
|---|---|
| **Kit Components** | (for 20 Tests) |
| 1. Reaction Mix (yellow) | 200 µl |
| 2. Buffer Solution (blue) | 1 ml |
| 3. Positive Template Control (red) | 20 µl |
| 4. Internal control DNA template (purple) | 20 µl |
| 5. Internal control primers mix (orange-gold) | 100 µl |

**Storage:** -20°C
Avoid repeated changes in the Reaction Mix temperature.
When in use, always keep the Reaction Mix on ice!

**Shipment:** 2-8°C (up to 27 days),
On dry ice for longer periods

**Additional reagents not included in the kit**

Molecular biology grade water, Agarose (e.g. Agarose Basic A8963, Agarose low EEO (Agarose Standard) A2114, Agarose MP A1091) and reagents for Agarose gel electrophoresis.

**Equipment required**

PCR thermocycler
Sterile microcentrifuge tubes
Agarose gel electrophoresis apparatus
Microcentrifuge
Micropipettes and sterile pipette tips (autoclaved)

Reference:

Rottem, S. & Barile, F.M. (1993) *TIBTECH* 11, 143-150

## Principle

Ribosomal RNA (rRNA) gene sequences of prokaryotes, including mycoplasmas, are well conserved, whereas, the lengths and sequences of the spacer region in the rRNA operon (for example, the region between 16S and 23S gene) differ from species to species. The detection procedure utilizing the PCR process with this primer set consists of:

1. Amplification of a conserved and mycoplasma-specific 16S rRNA gene region using two primers.
2. Detection of the amplified fragment by agarose gel electrophoresis.

This system does not allow the amplification of DNA originating from other sources, such as mammalian cells or bacteria, which affect the detection result. Amplification of the gene sequence with PCR using this primer set enhances not only the sensitivity, but also the specificity of detection.

## Protocol

### A. Sample preparation

Transfer 1.0ml cell culture supernatant into a 1.5ml sterile centrifuge tube. To pellet cellular debris, centrifuge the sample at 250xg briefly. Transfer the supernatant into a new sterile tube and centrifuge at 15,000-20,000xg for 10 minutes to sediment mycoplasma. Carefully decant the supernatant and keep the pellet (the pellet will not always be visible). Re-suspend the pellet with 50µl of the Buffer Solution supplied and mix thoroughly with a micropipette. Heat to 95°C for 3 minutes. The test sample can be stored at this stage at -20°C for later use

### B. PCR amplification

1. Test samples: Prepare the reaction mixture in a PCR tube by combining the reagents shown below:

| Reagents | Volume |
|---|---|
| $H_2O$ (for PCR) | 29 µl |
| Reaction Mix | 10 µl |
| Test sample | 5 µl |
| Internal control DNA template | 1 µl |
| Internal control primers mix | 5 µl |
| Total | 50 µl |

2. Negative control:in a separate PCR tube, use 5µl of sterile distilled H2O or the Buffer Solution supplied as test sample in the reaction mixture above.

3. Control DNA templates:prepare the reaction mixture in a separate PCR tube by combining the reagents shown below:

| Reagents | Volume |
|---|---|
| $H_2O$ (for PCR) | 33 µl |
| Reaction Mix | 10 µl |
| Internal control DNA template | 1 µl |
| Internal control primers mix | 5 µl |
| Positive control DNA | 1 µl |
| Total | 50 µl |

4. If required, overlay mineral oil (approximately 40µl) to avoid evaporation.

5. Place all tubes in a thermal cycler. Set the parameters for the following conditions and perform PCR

3. Place all tubes in DNA thermal cycler. Set the parameters for the following conditions and perform PCR.

| | | |
|---|---|---|
| 94°C | 30 secs. | |
| 94°C | 30 secs. | 36 cycles |
| 60°C | 120 secs. | |
| 72°C | 60 secs. | |
| 72°C | 5 min. | |
| 4 - 8°C | Cool down and hold | |

## C. Analysis of amplified products by Agarose gel electrophoresis

1. Apply 20 µl of the PCR product to the gel electrophoresis.
2. Perform Agarose gel electrophoresis with the PCR amplified samples to verify the amplified product and its size. Use 2 % Agarose gel.

   The size of DNA fragments amplified using the specific primers in this kit is **270 bp**.

   **Note**: When the system is very sensitive, annealed primer or primer dimers might be detected as smaller bands (20 - 50 bp).

## D. Control templates

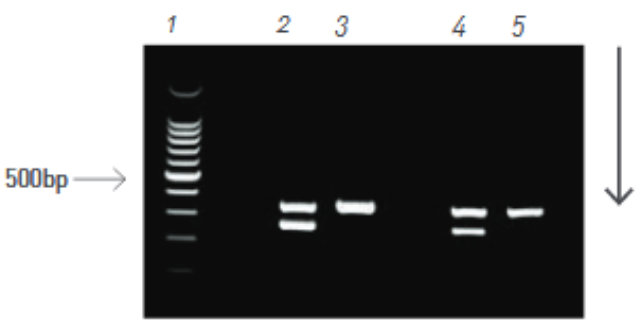
By the use of Positive Template Control, PCR efficiency can be checked. The size of the PCR product obtained using the positive template is 270bp. The use internal control is to check for PCR Inhibition by the test sample (false negative). The size of the PCR product obtained using the internal control template is 357bp

## E. Interpretation of results

1. Mycoplasma positive sample shows a 270bp band as well as 357bp band.
2. Mycoplasma negative sample shows a 357bp band only.
3. PCR inhibition yields no band.
4. Negative control: one band of 357bp.
5. Primer self-annealing may yield a band of <100bp in size. This does not affect the sensitivity and precision of the test

| Band at 270 Bp | Internal control band at 357bp | Interpretation |
|---|---|---|
| Positive | Irrelevant* | Mycoplasma positive sample |
| Positive | Negative | PCR inhibition (test not valid) |
| Negative | Negative | Mycoplasma negative sample |

*Note: If the mycoplasma concentration in the sample is high the Internal Control band might be absent due to competition




**Agarose gel of PCR products of different controls and samples:**

1. DNA size marker
2. Internal and Positive control
3. Negative control
4. Test sample: positive
5. Test sample: negative

# Additional Product Information

## A. Sensitivity. Detection limits of selected Mycoplasma species using this PCR test kit

| Species | minimum concentration of Mycoplasma detected |
|---|---|
| *M. fermentans* | 240 CFU/ml |
| *M. capricolum* | 110 CFU/ml |
| *M. penetrans* | 200 CFU/ml |
| *M. hyorhinis* | 210 CFU/ml |

## B. Comparing two different Mycoplasma detection methods

| PCR-Based Method | Microbiological Culture |
|---|---|
| Rapid | Time consuming |
| Results within 5 hours | Results require up to 3 weeks |
| Simple: one-step reaction.<br>Fast: less than 10 minutes to prepare samples for PCR.<br>Convenient: PCR master mix contains everything (incl. *Taq* DNA polymerase) | Cumbersome |
| For routine use in molecular biology labs | Requires trained personnel |
| Minimal sample handling reducing the risk of contamination | Enhanced contamination risk due to proliferation of Mycoplasma bacteria |
| Sensitive* | Sensitive* (100 – 1000 CFU/ml) |
| Easy to detect *M. hyorhinis* | *Very difficult detection of M. hyorhinis* |

*Adequate to diagnose cell cultures infected with Mycoplasma. Infections usually result in Mycoplasma titers of $10^5$ – $10^8$ CFU/ml (McGarrily, 1982)

## C. Related products:

| Code | Description |
|---|---|
| A8994 | PCR Mycoplasma Test Kit II - Lyophilized components, internal amplification control included. |
| A2114 | Agarose Low EEO (Agarose Standard) |
| A8963 | Agarose Basic |
| A1091 | Agarose MP |
| A4227 | TAE buffer (10X) for molecular biology |
| A3945 | TBE buffer (10X) for molecular biology |