

## Mycokit

CAT N°: K1000 (for 20 tests)

**Storage conditions :** Store at -20°C

Avoid repeated changes in the Reaction Mix temperature.

When in use, always keep the Reaction Mix on ice.

**Shelf life :** 36 months

**Kit composition :**

- PCR Reaction Mix 200µl
- Buffer Solution 1.0ml
- Positive Template Control 20µl

**Reagents not supplied in the kit :**

- Mineral oil
- Agarose gel
- Distilled sterilized water

**Equipment required :**

- Authorized thermal cycler for PCR
- Microcentrifuge tubes
- Agarose gel electrophoresis apparatus
- Microcentrifuge
- Micropipets and pipette tips (autoclaved)

**Recommended use :**

- Respect storage conditions of the product
  - Do not use the product after its expiry date
  - Store product in an area protected from light
  - Manipulate the test sample in aseptic conditions (e.g: under laminar air flow)
  - Wear clothes adapted to the manipulation of the product to avoid contamination (e.g: gloves, mask, hygiene cap, overall ...)
  - It is recommended to use the product immediately after its thaw out.
- The product is intended to be use in-vitro, in laboratory only. Do not use it in therapy, human or veterinary applications.

**Application :**

Mycokit is designed to detect the presence of mycoplasma contaminating biological materials, such as cultured cells.

Mycoplasma detection by the direct culture procedure is time-consuming and some mycoplasma species are difficult to cultivate.

With PCR testing, results are obtained within a few hours, since the presence of contaminant mycoplasma can be easily detected simply by verifying the bands of amplified DNA fragments in electrophoresis. There is no need to prepare probes labeled with radioisotopes, or to calculate enzyme, dNTP's or buffer concentrations. Instead, a ready-to-use, optimized PCR mix is supplied.

The reaction mix contains a precipitant for direct loading of PCR products onto agarose gel.

The primer set allows detection of various mycoplasma species (*M. fermentans*, *M. hyorhina*, *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.

**Principle :**

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 tissue samples 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. Amplified products are then detected by agarose gel electrophoresis.

**Instructions for use :**

-Test sample preparation:

Transfer 0.5-1.0ml cell culture supernatant into a 2ml centrifuge tube. To pellet cellular debris, centrifuge the sample at 250 x g briefly. Transfer the supernatant into a fresh sterile tube and centrifuge at 15,000-20,000 x g 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 and mix thoroughly with a micropipet. Heat to 95°C for 3 minutes. The test sample can be stored at this stage at -20°C for later use.

-PCR amplification:

Prepare the reaction mixture in a PCR tube by combining the reagents shown below:

Reagents	Volume
H2O	35µl
Reaction mix	10µl
Test sample	5µl

Overlay mineral oil (approximately 40µl) to avoid the evaporation of the reaction mixture.

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.  
60°C 120 secs.  
72°C 60 secs. } 35 cycles

94°C 30 secs.  
60°C 120 secs.  
72°C 5 min.

-Analysis of amplified products by gel electrophoresis:

- 1) Apply 20µl of the PCR product to the gel electrophoresis. Do not add loading buffer to the samples. Use 2% agarose gel.
- 2) Perform agarose gel electrophoresis with the PCR amplified samples to verify the amplified product and its size.

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

-Control template:

By the use of 1µl of Positive Template Control as a test sample, PCR efficiency can be checked. The size of the PCR product obtained using the positive template with primer pairs is 270bp.

**Reference :**

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