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 vials on ice.

Shelf life: 36 months

Kit composition :
- Reaction Mix 200µl
- Buffer Solution 1.0ml
- Positive Template Control 20µl
- Internal control DNA template 20µl
- Internal control primers mix 100µl

Reagents not supplied in the kit:
- Mineral Oil
- Agarose for gel electrophoresis
- Sterile distilled water

Equipment required:
- Thermal cycler for PCR and PCR tubes
- Microcentrifuge tubes
- Agarose gel electrophoresis apparatus
- Microcentrifuge
- Micropipettes 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 used in vitro for research or further manufacturing only and not for use as an Active Pharmaceutical Ingredient or food or animal feed.

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. 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.

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:

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

Reagents	Volume
H20 (for PCR)	29µl
Reaction mix	10µl
Test sample	5µl
Internal control DNA template	1µl
Internal control primers mix	5µl
Total	**50µl**

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
H20 (for PCR)	33µl
Reaction mix	10µl
Internal control DNA	1µl
Internal control primers mix	5µl
Positive control DNA	1µl
Total	**50µl**

4) Overlay mineral oil (approximately 40µl) to avoid the evaporation of the reaction mixture.
5) 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.

4°C hold

- 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 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.

- Interpretation of the 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 270bp	Internal control band at 357bp	Interpretation
Positive	Irrelevant	Mycoplasma positive sample
Negative	Negative	PCR inhibition (test not valid)
Negative	Positive	Mycoplasma negative sample

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

Reference:

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