BI
Biological Industries

EZ-PCR Mycoplasma Detection Kit

PCR Mix for the detection of mycoplasma in cell culture

With internal control

Cat. No.: 20-700-20 (for 20 tests)
Store at: -20°C

Instruction

EZ-PCR Mycoplasma Detection 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 EZ-PCR Mycoplasma Detection 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).

Kit Components

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

1. Thermal cycler for PCR and PCR tubes
2. Sterile microcentrifuge tubes
3. Agarose gel electrophoresis apparatus
4. Microcentrifuge
5. Micropipettes and sterile pipette tips

Storage

Store at -20°C
Avoid repeated changes in the Reaction Mix temperature.
When in use, always keep kit vials on ice!

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.

Instructions for use

A. Test 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)	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 H_2O 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)	33µl
Reaction Mix	10µl
Internal control DNA	1µl
Internal control primers mix	5µl
Positive control DNA	1µl
Total	**50µl**

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.

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

C. Analysis of amplified products by gel electrophoresis

It is not necessary to add loading buffer to the samples unless required. Use 2% agarose gel.

1. Carefully load 20µl of the PCR product into the gel for electrophoresis.
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 mycoplasma specific primers in this kit is 270bp.

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.

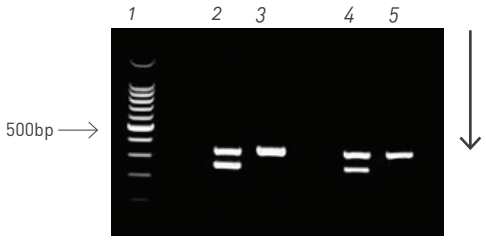
E. Interpretation of the results

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

A typical agarose gel



1. DNA size marker (100bp)
2. Internal and positive controls
3. Negative control
4. Test sample: positive
5. Test sample: negative

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