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 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 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.

As the Mycoplasma concentration in contaminated cultures is pretty high, samples may be taken even 1 day after the passage of the cells.

Product No.	**A3744,0020**
Kit Components	(for 20 Tests)
1. Reaction Mix	200 µl
2. Buffer Solution	1 ml
3. Positive Template Control	20 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
Microcentrifuge tubes
Agarose gel electrophoresis apparatus
Microcentrifuge
Micropipettes and pipette tips (autoclaved)

Reference:

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

Principle

rRNA gene sequences of prokaryotes, including Mycoplasma, 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 cultured 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. Amplified products are then detected by agarose gel electrophoresis.

Protocol

A. Sample preparation

Transfer 0.5 - 1.0 ml cell culture supernatant into a 2 ml centrifuge tube. To pellet cellular debris, centrifuge the sample at 250xg briefly. Transfer the supernatant into a fresh 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 50 µl of the Buffer Solution 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. Prepare the reaction mixture in a PCR tube by combining the reagents shown below:

Reagents	Volume
H_2O	35 µl
Reaction Mix	10 µl
Test sample	5 µl

2. *(Optional) Only if the PCR thermocycler is not equipped with a heated lid:* Overlay mineral oil (approximately 40 µl) to avoid the evaporation of the reaction mixture.

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	4 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. Positive Control

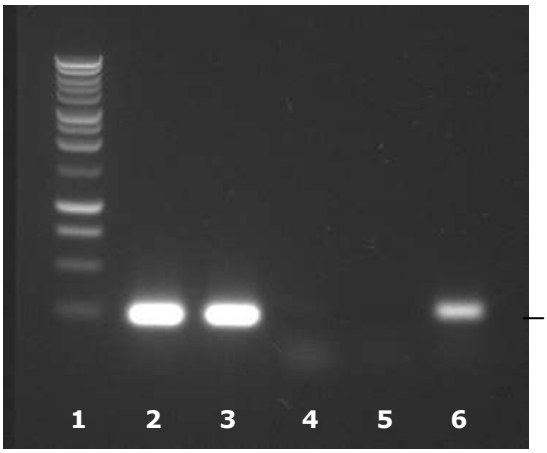
PCR efficiency can be checked by the use of 1 µl of Positive Template Control as a test sample. The size of the PCR product obtained using the positive template with primer pairs is 270 bp.

This PCR kit for Mycoplasma detection does not contain an internal control. Please see related products section below for more kit options. Optional: If you want an internal control to run with the tested sample (to check for PCR inhibition), you may use the positive template control with the tested sample in the same tube. Note that because the positive template control yields a 270 bp fragment (same as you get with Mycoplasma contaminated sample), you will have to run the tested sample alone as well.

E. Interpretation of results

PCR products of **270 bp** indicate the presence of Mycoplasma DNA.

Mycoplasma positive samples and Positive Template Control will result in a 270 bp product.
If the Positive Template Control gives no 270 bp product this indicates failure of the PCR. Check function of the thermocycler and double-check the PCR cycler program



Agarose gel of PCR products of different controls and samples:

1. DNA size marker
2. Positive Template Control
3. Positive Template Control in cell culture medium
4. Negative control (molecular biology grade water)
5. Negative control (buffer solution)
6. Mycoplasma positive sample

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. Fast: less than 10 minutes to prepare samples for PCR. 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
A9019	qPCR Mycoplasma Test Kit - For real-time/qPCR detection; internal amplification control included.
A9753	PCR Mycoplasma Test Kit - Lyophilized components, PCR master mix includes *Taq* DNA polymerase and internal amplification control.
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