

### EZ-PCR Mycoplasma Test Kit

Cat. #	Product Name	Size	Storage temp.
20-700-10	EZ-PCR Mycoplasma Test Kit	10 tests	-20°C
20-700-20		20 tests	

Ready-to-use PCR Mix for the detection of mycoplasma in cell culture

#### Introduction:

EZ-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 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. arthritis*, *M. bovis*, *M. pneumoniae*, *M. pirum* and *M. capricolum*), as well as *Acholeplasma* and *Spiroplasma* species, with high sensitivity and specificity.

#### Kit Components:

	20-700-10	20-700-20
Reaction Mix	100µl	200µl
Buffer Solution	0.5ml	1ml
Positive Template Control	10µl	20µl

Reagents not supplied in the kit:

1. Mineral Oil
2. Agarose gel
3. Distilled Sterilized water

#### Equipment required:

1. Authorized thermal cycler for PCR
2. Microcentrifuge tubes
3. Agarose gel electrophoresis apparatus
4. Microcentrifuge
5. Micropipets and pipette tips (autoclaved)

Storage: -20°C

Avoid repeated changes in the Reaction Mix temperature

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

#### Reference:

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

#### 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.

#### Protocol:

##### A. 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 -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 <sub>2</sub> O	35µl
Reaction Mix	10µl
Test sample	5µl

2. Overlay mineral oil (approximately 40µl) 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 seconds	
94°C	30 seconds	} 35 cycles
60°C	120 seconds	
72°C	60 seconds	
94°C	30 seconds	
60°C	120 seconds	
72°C	5 minutes	

**C. 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.

**D. 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.