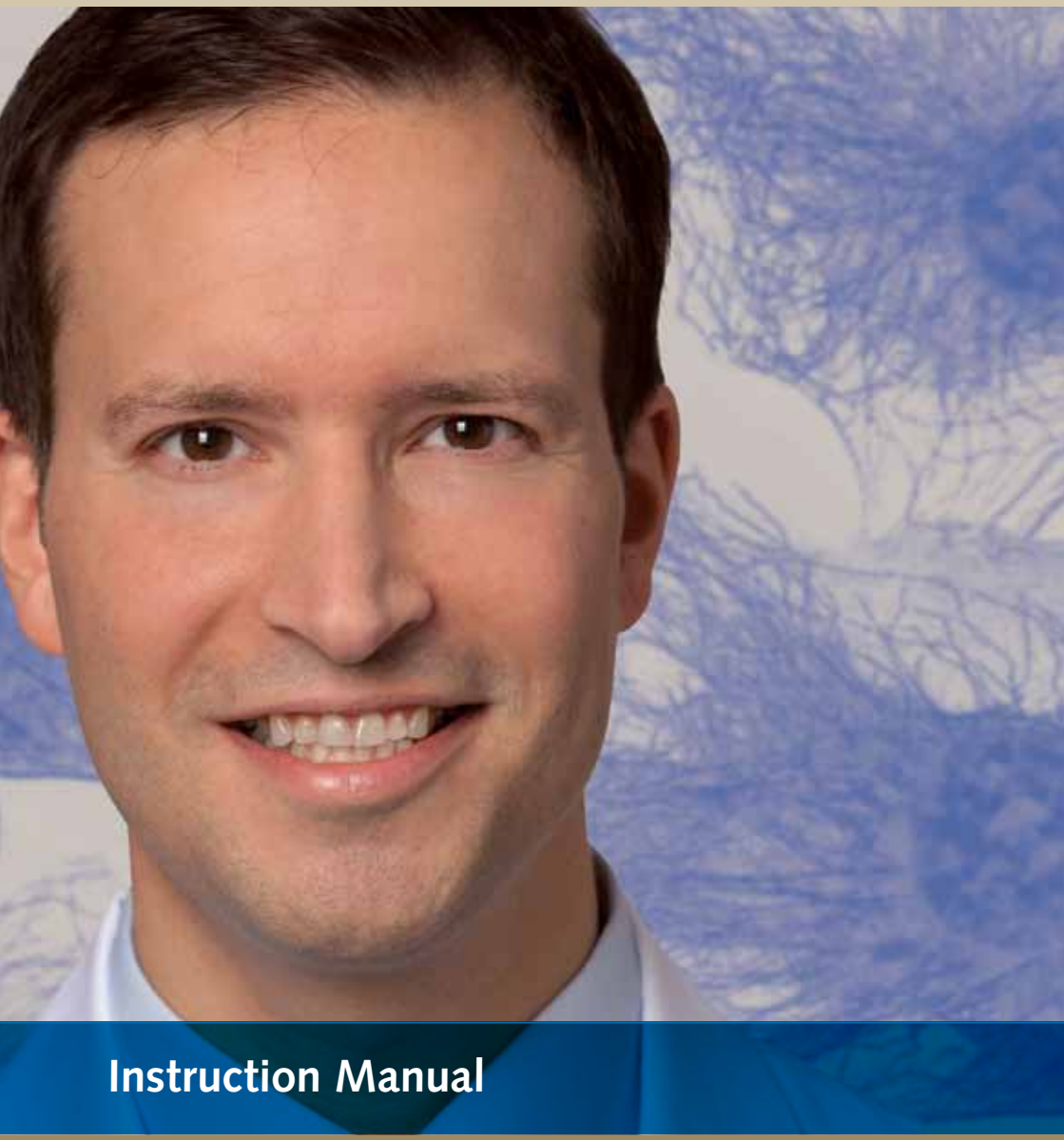
PCR Mycoplasma Test Kit II



Instruction Manual

PromoKine

Contents

Introduction

PromoKine's PCR Mycoplasma Test Kit II has been 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, sensitive and reliable results are obtained within a few hours, since the presence of contaminant mycoplasma can be easily detected by simply verifying the bands of amplified DNA fragments after gel 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 (including a high-performance Taq polymerase) is supplied. The reaction mix contains a precipitant for direct loading of PCR products onto an 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*.

Kit Components

	PK-CA20-700-20
	20 tests
Reaction Mix	200 µl
Buffer Solution	1 ml
Positive Template Control	20 µl

* A complete list of mycoplasma species that can be detected using our PCR Mycoplasma Test Kit II can be found in the Knowledge Base of our website.

Required Reagents and Equipment

Reagents not supplied in the kit

- Mineral oil (if using thermal cycler without heated lid)
- Agarose gel
- Distilled sterile water

Equipment required

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

Storage

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

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 the 16S and 23S genes) 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 by 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

Test 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 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 50 µl 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
H_2O	35 µl
Reaction Mix	10 µl
Test Sample	5 µl

2. If not using a thermal cycler having a heated lid, overlay mixture with mineral oil (approximately 40 µl) to avoid evaporation of the reaction mixture.
3. Place all tubes in a PCR thermal cycler. Set the parameters for the following conditions and perform the PCR.

94°C	30 secs.	
94°C	30 secs.	35 cycles
60°C	120 secs.	
72°C	60 secs.	
94°C	30 secs.	
60°C	120 secs.	
72°C	5 min.	

Analysis of amplified products by gel electrophoresis

1. Apply 20 µl of the PCR product onto an agarose gel for electrophoresis. It is not necessary to add loading buffer to the samples*. Use a 2% agarose gel.

Note: If you have overlayed your PCR sample with mineral oil, carefully pipette PCR product (lower phase) onto the 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 around 270 bp (depending on the mycoplasma species).

Control Template

To check PCR efficiency use 1 µl of the "Positive Template Control" as a test sample. The size of the PCR product obtained using the positive template with primer pairs is 270 bp.
*If desired, you may add 4 µl 1X Gel Loading Buffer containing a marker dye (e.g. bromophenol blue) to the samples.

1X Gel Loading Dye, Blue (6X):

- 2.5 % Ficoll 400
- 11 mM EDTA
- 3.3 mM Tris-HCl
- 0.017 % SDS
- 0.015 % Bromophenol Blue

Note: A combination of Positive Template Control+Test Sample may be used as an internal control for verifying a successful PCR run. This will validate that the test sample does not inhibit the PCR process.

Reference

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

Ordering Information

Product Name	Size	Catalog Number
PCR Mycoplasma Test Kit II	20 Tests	PK-CA20-700-20

For in vitro research use only.
Not for diagnostic or therapeutic procedures.

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