PCR Mycoplasma Detection Kit

Introduction

Mycoplasma is the smallest prokaryotic microorganism, only 0.1-0.3 μm in size. Due to their small size, mycoplasmas can penetrate rated filters (0.22~0.45 μm). Mycoplasma contamination remains a major problem in cell culture. Mycoplasmas can alter the DNA, RNA, and protein synthesis of culture cells, but they may not noticeably affect cell growth rates in many cases. Therefore, it is difficult to discover mycoplasma contamination with the naked eye. And mycoplasma detection should be performed regularly during cell culture.

There are many methods to detect mycoplasma contamination, such as the culture-based method, fluorescent staining method, ELISA and so on. However, most of these methods are time-consuming, complex to operate, and not highly sensitive. PCR-based detection is a relatively simple method within a few hours by PCR and electrophoresis. If necessary, PCR products can be sequenced to determine the species of contaminated mycoplasma.

The PCR Mycoplasma Detection Kit is a kit for the specific detection of mycoplasma by nested PCR. Nested PCR uses two pairs of primers to amplify the mycoplasma genome DNA, which is more sensitive than one-step PCR detection. As shown in Figure 1, the rRNA gene sequence of prokaryotes is very conserved, and the rRNA operon encodes the DNA of rRNA Space regions vary greatly between various biological species, such as the interval between 16S and 23S. Thus, a pair of F1/R1 primers can be designed on the conserved region DNA encoding 16S and 23S and can be used for amplification, which is the first round of PCR of nested PCR (1st PCR). 1st PCR can be used to initially determine whether there is mycoplasma contamination; An F2 primer designed on the conserved region of the DNA spacer region encoding 16S and 23S rRNA, and an R2 primer designed on the DNA encoding 23S rRNA are used for the second amplification of PCR (2nd PCR) for further confirmation. Compared with one-step PCR, nested PCR can greatly improve the specificity and sensitivity of detection.

The kit provides a rapid, efficient, and highly sensitive detection method for mycoplasma contamination, and Positive Control is provided to confirm that the kit is properly tested and whether the sample contains PCR inhibitors. If mycoplasma contamination is found after detection, the contaminated cells can be discarded directly. If cells are precious, consider treating the cells with Mycoplasma Remover Reagent.

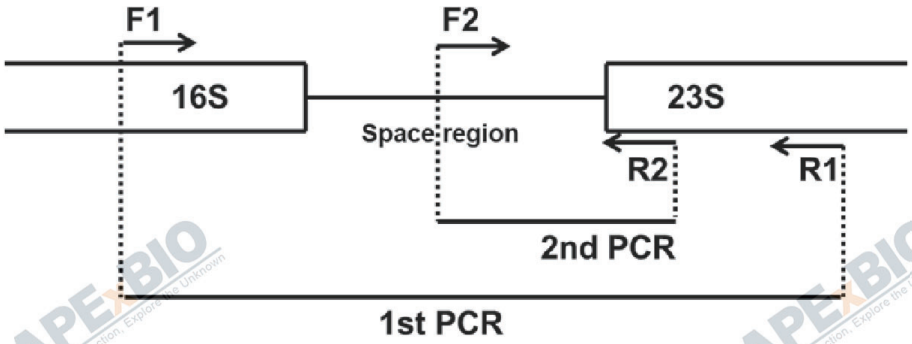

Figure 1: Principle of nested PCR-based mycoplasma detection

Components and Storage

Components	K2821-50 T	K2821-100 T
2×Taq PCR Master Mix (with dye)	1 mL	2 x 1 mL
1st PCR Primer Mix (50X)	20 μL	40 μL
2nd PCR Primer Mix (50X)	20 μL	40 μL
Positive Control	20 μL	40 μL

Store the kit at -20°C, avoiding repeated freeze and thaw cycles, stable for 1 year.

Protocol

1. **Sample preparation:** If cells are cultured with cell culture media, the culture supernatant cultured for 3-6 days can be taken directly as the detection sample. If a cell suspension is used, it is necessary to extract DNA and then perform the PCR reaction. If the sample contains PCR inhibitors, extract DNA and then perform the PCR reaction as well.

***Note:** To confirm whether the sample contains PCR inhibitors, a positive control experiment using the Positive Control can be performed.

2. **1st PCR:**

1) Advance thaw the various solutions required for the PCR reaction, while 2×Taq PCR Master Mix (with dye) needs to be thawed in an ice bath. Prepare the reaction mixture shown below in the ice bath:

Reagents	Final concentration	Volume	Volume
Ultrapure water	-	7.6-9.2 μL	19-23 μL
Simple or Positive Control	0.2 pg/μL -20 ng/μL	0.4-2 μL	1-5 μL
1st PCR Primer Mix (50X)	1X	0.4 μL	1 μL
2×Taq PCR Master Mix (with dye)	1X	10 μL	25 μL
Total Volume	-	20 μL	50 μL

***Note:** The recommended volume for Positive Controls is 1 μL. At the same time, a negative control without the template can be set up to determine whether the sample is really contaminated with mycoplasma or a false positive.

2) Perform the PCR reaction under the following conditions:

PCR Condition	Temp	Time
Step 1 (Initial denaturation)	95°C	30 s
Step 2 (Denaturation)	95°C	30 s
Step 3 (Annealing)	58°C	30 s
Step 4 (Extension)	72°C	1 min
Step 5 (X Cycles)	Go to Step2 for 27-35 cycles	
Step 6 (Final extension)	72°C	5 min
Step 7 (Holding)	4°C	Forever

3. **2nd PCR：**

1) Advance thaw the various solutions required for the PCR reaction, while 2×Taq PCR Master Mix (with dye) needs to be thawed in an ice bath. Prepare the reaction mixture shown below in the ice bath:

Reagents	Final concentration	Volume	Volume
Ultrapure water	-	9.4 μL	23.5 μL
1st PCR product	0.2 pg/μL -20 ng/μL	0.2 μL	0.5 μL
2nd PCR Primer Mix (50X)	1X	0.4 μL	1 μL
2×Taq PCR Master Mix (with dye)	1X	10 μL	25 μL
Total Volume	-	20 μL	50 μL

2) Perform the PCR reaction under the following conditions:

PCR Condition	Temp	Time
Step 1 (Initial denaturation)	95°C	30 s
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Step 4 (Extension)	72°C	1 min
Step 5 (X Cycles)	Go to Step2 for 27-35 cycles	
Step 6 (Final extension)	72°C	5 min
Step 7 (Holding)	4°C	Forever

4. **Gel electrophoresis:** After the reaction, separately take 10 μL 1st PCR and 2nd PCR products to perform the electrophoresis. If only to confirm the mycoplasma contamination, 1-2% agarose gel is suitable. If need to confirm the fragment size of PCR products, 2% agarose gel is preferred.

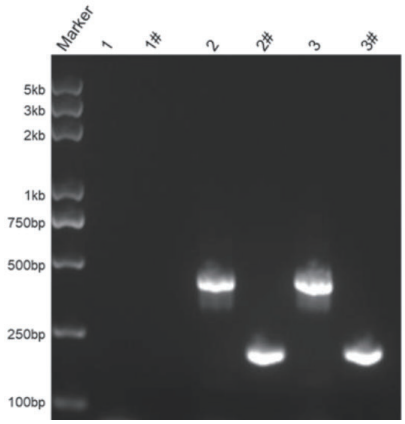

Figure 2: Schematic of agarose gel electrophoresis of 1st PCR and 2nd PCR products.

1, 2, 3 are 1st PCR products; 1#, 2#, 3# are the 2nd PCR products. The templates for each lane are: 1 and 1#, negative cell

supernatant; 2 and 2#, mycoplasma contaminated cell supernatant; 3 and 3#, Positive Control.

5. **About Positive Control：**

   1) First, the Positive Control can be used to determine whether the PCR reaction is working properly. At the same time, Positive Control can also be added to the sample to determine whether the sample contains PCR inhibitors. The Positive Control is a synthetic DNA fragment, while its 1st PCR and 2nd PCR products are 448 bp and 211 bp, respectively.

   2) If the Positive Control is not amplified by electrophoresis when used alone, it indicates that the PCR reaction does not work well.

   3) If the expected amplification fragment appears in the electrophoresis when the positive control is used alone, but there is no amplification fragment for the sample containing Positive Control. This indicates that the PCR reaction is no problem, but there are PCR inhibitors in the sample. At this point, the sample can be extracted and then the extracted DNA can be used for the PCR reaction. It is also possible to consider diluting the sample with ultrapure water or PBS before performing PCR. At this time, if the mycoplasma contamination in the sample is serious, the dilution will basically not affect the detection results; but if the mycoplasma contamination in the sample is slightly, the dilution may reduce the detection sensitivity.

   4) When PCR is added to the sample, electrophoresis may obtain only the PCR amplification product of mycoplasma or only the amplification product of the positive control, and it is also possible to obtain both amplification products at the same time. Because if one of the templates is excessive, it is usually easier to be amplified and detected by electrophoresis; At the same time, another template may not detect significant amplification.

Note

1. Since the PCR reaction is very sensitive, care needs to be taken when using Taq enzymes in experiments to avoid contamination of trace amounts of amplified DNA. At the same time, a negative control without a template can be set up to determine whether the sample is really contaminated with mycoplasma or a false positive. It is best to perform the experiment in a standard PCR laboratory as far as possible to avoid contamination.
2. It is recommended to use a tip with a filter element to perform the PCR reaction to minimize false positives.
3. It is recommended to prepare the PCR reaction with a dedicated pipette.
4. Wear a disposable mask throughout the experiment and avoid talking as much as possible to avoid mycoplasma contamination in saliva.
5. Generally, 1st PCR can initially identify the presence of mycoplasma contamination, but 2nd PCR is recommended for further confirmation.

6. If mycoplasma contamination is indeed present after detection, the contaminating cells can generally be discarded directly. However, if cells are precious, consider using Mycoplasma Remover Reagents (catalog number: C7201) to eliminate contamination. Meanwhile, use Mycoplasma Prevention Reagent (catalog numbers: C7202 or C7203) in routine cell culture.
7. This kit cannot detect human *Mycoplasma pneumoniae*.
8. For your safety and health, please wear lab coats and gloves during the experiment.
9. For research use only. Not to be used in clinical diagnostic or clinical trials.

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