Mycoplasma Detection Kit Data sheet

Kit Description:

Mycoplasma detection kit is designed for the identification of mycoplasma-contaminated cell culture. Mycoplasma infection can alter the infected cell at molecular level, cell growth rate, and morphology. Contamination directly affect the accuracy and reproducibility of experiments. This assay kit allows detection of various mycoplasmas species with high degree of sensitivity.

Kit Components:

- Taq polymerase	25 µl
- 2X Pre-Mix Buffer	270 µl
- Primer Set Mix	45 µl
- Positive Control	45 µl
- ddH_2O	200 µl

Reagents not supplied in the Kit:

- Agarose Gel
- Distilled Sterilize Water

Storage

Store at -20℃

Avoid repeated freeze-thaw cycles of the Kit to retain the maximum activity.

When in use, always keep the reaction mix on ice.

Sample preparation

1. The contaminated cell should remain in culture for at least 24-72 hour.

2. Collect 1 mL target culture medium into a 1.5 mL micro-centrifuge tube and centrifuge it for 5 mins at 2000g to pellet cells and debris. Use the supernatant as the test sample. The test sample can be stored at -20℃ for later use.

3. Setting the reactions according to the following table:

Component	Sample	Positive Control	Negative Control
Taq Polymerase	1 µl	1 µl	1 µl
Pre-Mix Buffer	12.5 µl	12.5 µl	12.5 µl
Primer mix	2 µl	2 µl	2 µl
Sample	2 µl	0 µl	0 µl
Positive Control	0 µl	2 µl	0 µl
ddH_2O	7.5 µl	7.5 µl	9.5 µl
Final Volume	25 µl	25 µl	25 µl

4. Set the parameters as following table and perform PCR:

Step	Temperature	Duration Time	Cycles
Activation	95℃	5min	1
Denaturation	95℃	30 secs	30-40 Cycles
Annealing	55℃	30 secs	
Extension	72℃	60 secs	
Extra-extension	72℃	10 min	1
Holding	4℃	-	-

5. Analysis of amplified products by gel electrophoresis, and the presence of PCR products at 450~550 bp in length (multiple bands is possible) indicates that the tested cell culture is contaminated with mycoplasmas.

Recommendations for Optimal Results

1. Reagents of the mycoplasma detection kit are mycoplasma free. Please do not utilize any other PCR reagents to perform the PCR.

2. Make aliquots of the reagents to avoid contamination and repeated freeze-thaw cycles.