PCR Mycoplasma Detection Kit

TOKU-E Product Data Sheet

PRODUCT INFORMATION

Product Name: PCR Mycoplasma Detection Kit
Item Number: M034
Appearance: Liquid

Storage temp: -20°C. Avoid repeated freeze-thaw cycles of the Mastermix.

Shelf life: All kit components are stable for 1 year from the date of shipping if stored and handled properly.

Kit Components

Component Name	Volume
2X PCR Taq Mastermix	1.25 ml (100 rxns)
Mycoplasma PCR Primer Mix	100 µl
Mycoplasma Positive Control	250 µl
Nuclease-Free H_20	1 ml

DESCRIPTION

The PCR Mycoplasma Detection Kit allows the identification of *Mycoplasma*-contaminated cell culture samples. *Mycoplasma* infection can induce cellular changes, including chromosome aberrations, changes in cell morphology and cell growth. This kit minimizes the risk for false positives while maintaining a high degree of specificity and sensitivity for over 200 strains of *Mycoplasmas* in < 1 hour.

METHOD

The supernatant is obtained from routine cell cultures. PCR Amplification targeting *Mycoplasma* DNA in the supernatant reveals the presence or absence of *Mycoplasma* in the cell culture. A no-template-control (NTC) reaction confirms the lack of contamination, while a positive control ensures that no PCR inhibitor interferes with the reaction. The Mastermix contains gel loading dye, making it convenient for gel electrophoresis.

Protocol

1. The prospective contaminated cells should be in culture for at least 48-72 hours prior to screening and at least 80% confluent. Collect 2.5 µl directly from the culture.

2. Mix individual components before use and assemble reaction on ice.

3. Prepare the reaction according to the Table below:

Component	Volume
2X PCR Taq Mastermix	12.5 µl
Mycoplasma PCR Primer Mix	1 µl
Test Sample, Positive control, or NTC	2.5 µl
Nuclease-free H_20	Up to 25 µl

4. Gently mix the reaction and briefly centrifuge. Perform 30-40 cycles of PCR amplification as follows:

Step	Temp	Duration	Cycles
Enzyme activation	95°C	3 min	1
Denaturation	95°C	15 s	30-40
Annealing	55°C	15 s	
Extension	72°C	15 s	
Final extension	72°C	1 m	1
Hold	4°C	-	-

5. After PCR, maintain the reaction at 4C° or store at -20°C until use.

6. Analyze the amplification products by agarose gel electrophoresis and visualize by ethidium bromide.

Notes: The presence of PCR products approximately 500 bp in length indicates the presence of *Mycoplasma* contamination in the original culture. Depending on the different *Mycoplasma* type, the length of the PCR product will vary between 370-550 bp.

Recommendations

- The Mastermix is optimized for *Mycoplasma* DNA amplification and has high resistance against any possible PCR inhibitors in the culture medium. Please do not use any other PCR reagents to perform PCR other than the materials in this kit.
- Make aliquots of the reagents to avoid contamination.
- Start the PCR as soon as the reaction mixture is prepared and always keep the reaction mixture on ice prior to PCR.

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