

ab289834 – Mycoplasma PCR Detection Kit

PCR based kit to detect mycoplasma in cell culture media. For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

<http://www.abcam.com/ab289834>

Storage and Stability

Upon arrival, the kit should be stored at -20°C. Avoid repeated freeze-thaw cycles of the MasterMix to retain maximum performance.

Materials supplied

Item	Quantity	Storage condition
ExpressTaq 2X PCR MasterMix	1.25 mL	-20°C
Mycoplasma PCR Primer Mix	100 µL	-20°C
Mycoplasma Positive Control	250 µL	-20°C
Nuclease-free water	1 mL	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Thermal Cycler
- PCR tubes
- Nuclease-free water
- 1.5 ml microcentrifuge tubes
- Reagents and equipment for agarose gel electrophoresis
- Ethidium bromide or Safe Image™ Basic DNA Stain
- DNA ladder

PCR Detection Protocol

1. Collected samples can be stored at 4°C for 3-5 days before testing. The cells should remain in culture for at least 48-72 hours undisturbed prior to screening for the presence of mycoplasmas and should be at least 80% confluent. Alternatively, the supernatant can be frozen and thawed before testing provided that the supernatant was in culture 48-72 hours before testing. Collect 2.5 µL directly from culture.
2. Mix individual components before use and assemble reaction on ice.

Components	Test Samples	Positive Control	Negative Control
ExpressTaq 2X PCR MasterMix	12.5 µL	12.5 µL	12.5 µL
Mycoplasma PCR Primer Mix	1 µL	1 µL	1 µL
Test sample	2.5 µL	-	-
Mycoplasma Positive Control	-	2.5 µL	-
Nuclease-free water	9 µL	9 µL	11.5 µL
Final volume per reaction	25 µL	25 µL	25 L

3. Gently mix the reaction and briefly centrifuge. Run thermocycling conditions for standard PCR.
4. Perform 30-40 cycles of PCR as follows:

Step	Temperature	Duration	Cycle(s)
Enzyme Activation	95°C	3 min	1
Denaturation	95°C	15 sec	30-40
Annealing	55°C	15 sec	30-40
Extension	72°C	15 sec	30-40
Final extension	72°C	1 min	1
Holding	4°C	-	-

5. After PCR, maintain the reaction at 4 °C or store at -20 °C until use.
6. Analyze the amplification products by agarose gel electrophoresis.
7. Visualize by Safe Image™ Basic DNA Stain or ethidium bromide staining.
8. The presence of PCR product of ~500 bp in length indicates that the cell culture tested is contaminated with mycoplasma. Note that the length of the PCR product will vary between 370-550 bp depending on different mycoplasma species/strains.

Recommendations for Optimal Results:

Always keep the reaction mixture chilled on ice prior to running the PCR and start the PCR as soon as the reaction mixture is prepared.

Technical Support

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