Mycoplasma PCR Detection Kit

Cat. No. G238

Store at -20°C.

Product Description

abm's **Mycoplasma PCR Detection Kit** offers **highly specific and sensitive** detection of 200+ strains of Mycoplasma in less than 2 hours. This best-selling kit is designed to minimize false positives, ensuring quick and reliable routine screening of cell cultures. Mycoplasma species are highly undesirable, easily acquired, and notoriously difficult to detect, altering infected cells at the molecular level and leading to visible changes in cell morphology and growth characteristics. Timely detection of Mycoplasma in cell cultures is recommended to deter wide-spread contamination and save on the costly efforts of elimination. Our proprietary MasterMix formulation contains a gel loading dye, making it convenient for gel electrophoresis.

Product Component	Quantity	Part No.
BlasTaq™ 2X PCR MasterMix	100 rxn (1.25 ml)	P895-1
Primer Mix	100 µl	P238-2
Positive Control	250 µl	P238-3
Nuclease-Free H_2O	1.0 ml	P100

Protocol

1. Cells should remain in culture for at least 48-72 hours undisturbed prior to screening and be at least 80% confluent.
2. From the cell culture, collect 2.5 µl of the media.
3. Mix individual components before use and assemble reaction on ice.

Component	Volume
BlasTaq™ 2X PCR MasterMix	12.5 µl
Primer Mix	1 µl
Test Sample, Positive Control, or NTC (ddH_2O)	2.5 µl
Nuclease-Free H_2O	Up to 25 µl

4. Gently mix the reaction components, and briefly centrifuge. Keep reaction mixture on ice prior to running the PCR and start the PCR as soon as the reaction mixture is prepared. Run thermocycling conditions for standard PCR:

Step	Temperature	Time	Cycle(s)
Initial Denaturation	95°C	3 min	1
Denaturation	95°C	15 sec	30 - 40
Annealing	55°C	15 sec	
Extension	72°C	15 sec	
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 on a 2% agarose gel. Visualize by ethidium bromide or SafeView™ Classic (Cat. No. **G108**) staining.
7. A PCR product **between 370-550 bp** in length indicates that the cell culture sample tested is contaminated with Mycoplasma. Note that the length of the PCR product will vary depending on the contaminating strain or species. Any bands that appear above or below that range are considered non-specific amplifications. The Positive Control sample should have a PCR product band around 500 bp in length.

General Notes

- Positive PCR detection of Mycoplasma will vary in length between 370-550 bp depending on the different strain or species detected. Non-specific amplifications outside of this range can be eliminated through Touchdown PCR. A touchdown protocol starts with higher annealing temperatures on the first 10 cycles then ramps down to the normal annealing temperature for the remainder of the cycles.

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