Mycoplasma Pro PCR Detection Kit

Cat. No. G239

Store at -20°C.

Product Description

Mycoplasma Pro PCR Detection Kit offers **highly specific and sensitive** detection of 300+ strains of Mycoplasma in less than 2 hours. This is an improved version of our best-selling kit (Cat. No. G238) which now includes:

- a modified Pro Primer Mix and more stringent cycling conditions to include additional Mycoplasma and Acholeplasma strains such as *A. laidlawii*
- an internal PCR control that helps confidently distinguish between a genuine negative and a false negative result due to technical errors or PCR inhibition.

Timely detection of Mycoplasmas in cell cultures is recommended to deter wide-spread contamination and save on the costly efforts of elimination. This kit includes a PCR MasterMix containing gel loading dye for added convenience.

Product Component	Quantity	Part No.
BlasTaq™ 2X PCR MasterMix	100 rxn (1.25 ml)	P895-1
Pro Primer Mix[1]	100 µl	P239-2
Positive Control	250 µl	P238-3
Nuclease-Free H_2O	1.0 ml	P100

[1] Includes internal PCR control primers and template.

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
Pro 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	60°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. The internal PCR control is a **180 bp** PCR product which should be present in all samples and eliminates false negatives due to PCR inhibition or user error. If PCR inhibition is noted, dilute the media sample with Nuclease-Free H_2O until the internal PCR control band is clearly seen (recommended dilution range 1/10 to 1/100).
8. A PCR product **between 300-600 bp** in length indicates that the cell culture sample tested is contaminated with Mycoplasma or Acholeplasma. Note that the length of the PCR product will vary depending on the contaminating strain or species. The Positive Control sample should have a strong PCR product band around 350-400 bp in length.

Applied Biological Materials Inc.● 1-866-757-2414 ● info@abmGood.com ● www.abmGood.com