Mycoplasma PCR Detection Kit

Store at -20°C

Cat. No.	Product Name	Quantity
G238	Mycoplasma PCR Detection Kit	100 reactions

Product Description

The Mycoplasma PCR Detection Kit allows for quick and reliable screening of cell cultures for mycoplasma contamination. Highly undesirable, easily acquired and notorious for an elusive onset of infection, mycoplasmas tend to alter the infected cells at a molecular level, ultimately leading to visible changes in cell morphology and growth characteristics. Timely detection of mycoplasmas in cell cultures is desirable in order to deter wide-spread contamination and save on the costly efforts of elimination.

Designed to perform with high specificity and sensitivity, **abm**'s Mycoplasma PCR Detection Kit helps minimize false positives while ensuring coverage over 200 species/strains of mycoplasmas with a quick protocol for routine screening.

Kit Components:

Product Components	Volume	Part No.
2X PCR Taq MasterMix	1.25 ml	G238-1
Mycoplasma PCR Primer Mix	100 µl	G238-2
Mycoplasma Positive Control	100 µl	G238-3
Nuclease-free H_2O	1 ml	RT-0

Shipping and Storage

Upon arrival, the Mycoplasma PCR Detection Kit should be stored at -20°C. Avoid repeated freeze-thaw cycles of the MasterMix to retain maximum performance. All kit components are stable for 1 year from the date of shipping, if stored and handled properly.

Protocol Overview

For screening, a small aliquot of the cell culture medium is spun at a high speed to sediment any contaminating mycoplasma, then applied as a template (Test sample) in a PCR after careful resuspension of the pellet. In parallel, a No-Template-Control (NTC) reaction is set-up as a negative control to rule out the possibility of any other source of contamination (there should be no amplification in this reaction). The set-up of a positive control reaction helps assess whether the PCR itself ran un-hindered. Targeted amplification of Mycoplasma DNA from the test sample confirms the presence of mycoplasmas in the cell culture while lack of amplification indicates absence of contamination.

Protocol

1. The cells should remain in culture for at least 48-72 hours prior to screening for the presence of mycoplasmas. Only collect the media sample once the cells have reached at least 80% confluence.

2. Withdraw 0.5 ml of cell culture medium and centrifuge it briefly at 2000 g to pellet cells/debris. Transfer the supernatant into a fresh sterile tube and centrifuge at 15,000-20,000 g for 10 minutes to sediment mycoplasma. Carefully remove 450 µl of the supernatant from this step and resuspend the pellet (may not always be visible) with the remaining 50 µl of medium. The resuspended solution will serve as the Test sample for PCR.

3. Set-up the various reactions according to the table below:

Component	Test Sample	Positive Control	Negative Control
2X PCR Taq 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	-	1µl	-
Nuclease-free H_2O	9 µl	10.5 µl	11.5 µl
Final volume per reaction	25 µl	25 µl	25 µl

4. Perform 30 - 40 cycles of PCR as follows:

Step	Temperature	Duration	Cycle(s)
Enzyme activation	95°C	5 mins	-
Denaturation	95°C	30 secs	30 - 40
Annealing	55°C	30 secs	
Extension	72°C	60 secs	
Final extension	72°C	10 mins	1
Holding	4°C	-	-

5. Resolve the amplification products by agarose gel electrophoresis and visualize by SafeView™ (Cat. No. **G108**) or ethidium bromide staining. For confirmation of fragment (DNA) length, **abm** recommends using 100 bp Plus OptiDNA Marker (Cat. No. **G193**).

6. The presence of PCR product approximately 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 the different mycoplasma species/strains.

Recommendations for Optimal Results

- The 2X PCR Taq MasterMix included in this kit is optimized for mycoplasma DNA amplification with high tolerance to potential PCR inhibitors from the culture medium. Therefore, do not employ any other PCR reagents (i.e. which are not part of the kit) to perform the PCR.
- Make aliquots of the reagents to avoid contamination and repeated freeze-thaw cycles.
- 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.

All **abm** PCR, RT-PCR, and qPCR products are ISO 13485:2003 and13485:2012 certified as diagnostic grade and in compliance with all regulatory requirements for the design and manufacture of medical devices, as outlined by the International Organization for Standardization (ISO). For technical questions, please email us at technical@abmgood.com or visit our website at www.abmGood.com.