Mycoplasma qPCR Detection Kit

Cat. No. G240

Store at -20°C.

Product Description

abm's Mycoplasma qPCR Detection Kit enables rapid detection of Mycoplasma and Acholeplasma species directly from cell culture supernatant—**no DNA extraction, sample preparation or gel electrophoresis required**. Designed for **maximum sensitivity**, the kit can reliably detect as few as 10 copies of Mycoplasma DNA, ensuring early and accurate detection of cell culture contamination.

Delivering **results in under 2 hours** with **complete elimination of non-specific signals**, this highly specific and robust assay is ideal for routine screening and quality control in any cell culture workflow. The kit is optimized for ease of use and is compatible with a broad range of real-time PCR instruments, providing researchers with a **fast, reliable, and cost-effective** tool for safeguarding cell culture integrity.

Product Component	Quantity	Part No.
MegaFi™ Pro 2X qPCR MasterMix	1.25 ml	P871-1
Primer Mix	100 µl	P240-2
Positive Control	50 µl	P240-3
ROX Reference Dye	15 µl	P101
Nuclease-Free H_2O	2 x 1.0 ml	P100

Protocol

MasterMix contains dye comparable to SYBR™ Green and EvaGreen®. ROX Reference Dye is provided separate from the MasterMix, making this kit universally compatible with most qPCR instruments.

*See **Rox Machine Compatibility** on our product page under the Documents tab on our website.*

The recommended amount of ROX Reference Dye to be added into the MasterMix may vary depending on the qPCR machine type:

- No ROX equipment: Not needed.
- Low ROX equipment: 1 µl/1.0 ml MasterMix.
- High ROX equipment: 10 µl/1.0 ml MasterMix.

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 5 µL of culture media and dilute with 20 µL of Nuclease-Free water (1/5 dilution). Dilution is needed to ensure there is no inhibition of the reaction.
3. Mix the following components before use. Assemble reactions on ice, set up each sample in duplicate, and include a No Template Control (NTC) and the provided Positive Control in each run.

Component	Volume
MegaFi™ Pro 2X qPCR MasterMix	10 µl
Primer Mix	1 µl
Diluted Sample, NTC, or Positive Control	2 µl
Nuclease-Free H_2O	Up to 20 µl

4. qPCR cycling conditions:

Step	Temperature	Duration	Cycle(s)
Enzyme Activation	95°C	10 min	1
Denaturation	95°C	30 sec	40
Annealing/Extension	63°C	45 sec	
Melting Curve	Consult instrument manual for model specific settings		

Results Interpretation

Sample	CT Value	Result
Test Sample	< 35	Positive
Test Sample	≥ 35	Negative
Positive Control	20-23	Valid Test
NTC	Any amplification	Invalid Test

Replicates should be closely matched, with Ct values within one cycle of each other. If amplification curves are unclear or unexpected, melt curve analysis may be used as an additional check to assess specificity.

Additional Notes

Due to the high sensitivity of the kit, follow these steps to prevent cross-contamination:

- Include a No Template Control (NTC) in every experiment to monitor contamination. Ideally NTCs should be set up first prior to sample handling.
- Ensure Positive Control material is handled last and kept physically separate from test samples. If possible, positive control should be set up in a separate room.
- If unexpected amplification occurs in negative controls, repeat the run with freshly prepared reagents and clean equipment.
- UV-sanitize lab and clean surfaces and pipettes with 10% Bleach.
- Use filtered, aerosol-resistant pipette tips and change tips between every sample and reagent.

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