MycoDetect - Mycoplasma qPCR Detection Kit
Instructions For Use

MYCO-100 100 Reactions

Manual Date: April 2025

Version Number: 2.0

Location: Microzone,
Suite 3, Faraday House,
King William Street,
Stourbridge,
DY8 4HD

T: +44(0)1384 444585

E: info@microzone.co.uk

W: microzone.co.uk

Contents

Introduction

This kit is for the identification of *Mollicutes* species nucleic acids using Real-Time PCR (qPCR). It targets specific conserved 16S-rDNA region of the of the *Mycoplasma*'s genome. It is intended for research use only by trained laboratory staff.

Kit Contents

Product	Product Code	Quantity
2X MegaMix Platinum Probe qPCR Mastermix	2MMPU-1	1 x 1 mL
Mycoplasma Detection Mix	MD-MYCO-0.5	1 x 500 µL
Positive Control	PC-MYCO-0.1	1 x 100 µL
Just Water	5JWA-1	1 x 1 mL

Storage and Stability

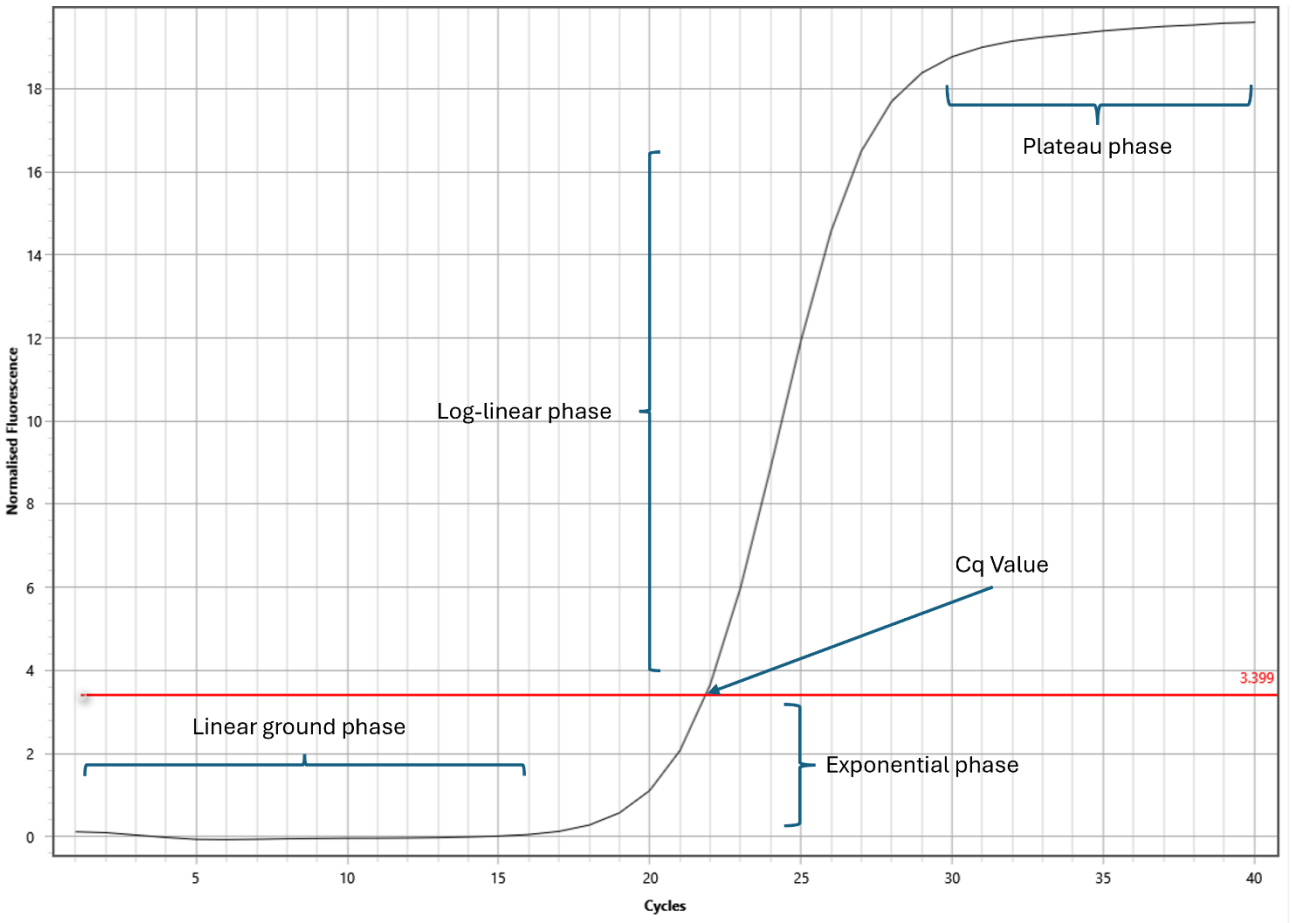
Store the MycoDetect - Mycoplasma qPCR Detection Kit at -20°C until the expiry date displayed on the label.

Materials Required but Not Provided

- Disposable powder-free nitrile gloves
- Pipettes (adjustable) and sterile filtered pipette tips
- 0.5/1.5 mL nuclease free microcentrifuge tubes
- Benchtop centrifuge
- Vortex mixer
- Decontaminated bench
- Optically clear PCR tubes and caps or 96-well microplate suitable for use with chosen PCR instrument
- Plate sealer (if required)
- qPCR Thermal cycler

Assay Principle

The assay utilises qPCR to detect the presence of specific sequences of DNA found in target of interest. Specifically, the dual-labelled hydrolysis probe technology is employed as a method of detection as it allows the sensitive and specific detection of multiple targets within one reaction. The qPCR amplification consists of 4 phases throughout the run: initially it is a linear ground phase, where there is not sufficient amplified DNA for a change in florescence to be measured; next is an exponential phase, where detectable fluorescence is observed; followed by a Log-linear phase, where the efficiency of the reaction is measured; finally the reaction reaches the plateau phase, where the reaction slows down resulting in a reduced rate of increase in fluorescence as the reaction components deplete.



Linear plot of a qPCR curve.

Technology

Dual-labelled hydrolysis probes are the most widely employed method in quantitative PCR (qPCR) for detecting and quantifying specific DNA sequences. It utilises a fluorescently labelled probe that binds to a complementary target sequence within the PCR product. The probe is a short oligonucleotide, consisting of a reporter dye at the 5’ end and a quencher at the 3’ end. While the probe is intact, the quencher suppresses the fluorescence of the reporter. During the PCR amplification, the 5' to 3' exonuclease activity of Taq polymerase cleaves the probe’s nucleotides, separating the reporter from the quencher, leading to an increase in fluorescence proportional to the amount of product amplified. The increase of fluorescence signal due to the dual-labelled hydrolysis probe technology allows the real-time monitoring of PCR amplification, providing highly specific and sensitive detection of nucleic acids.

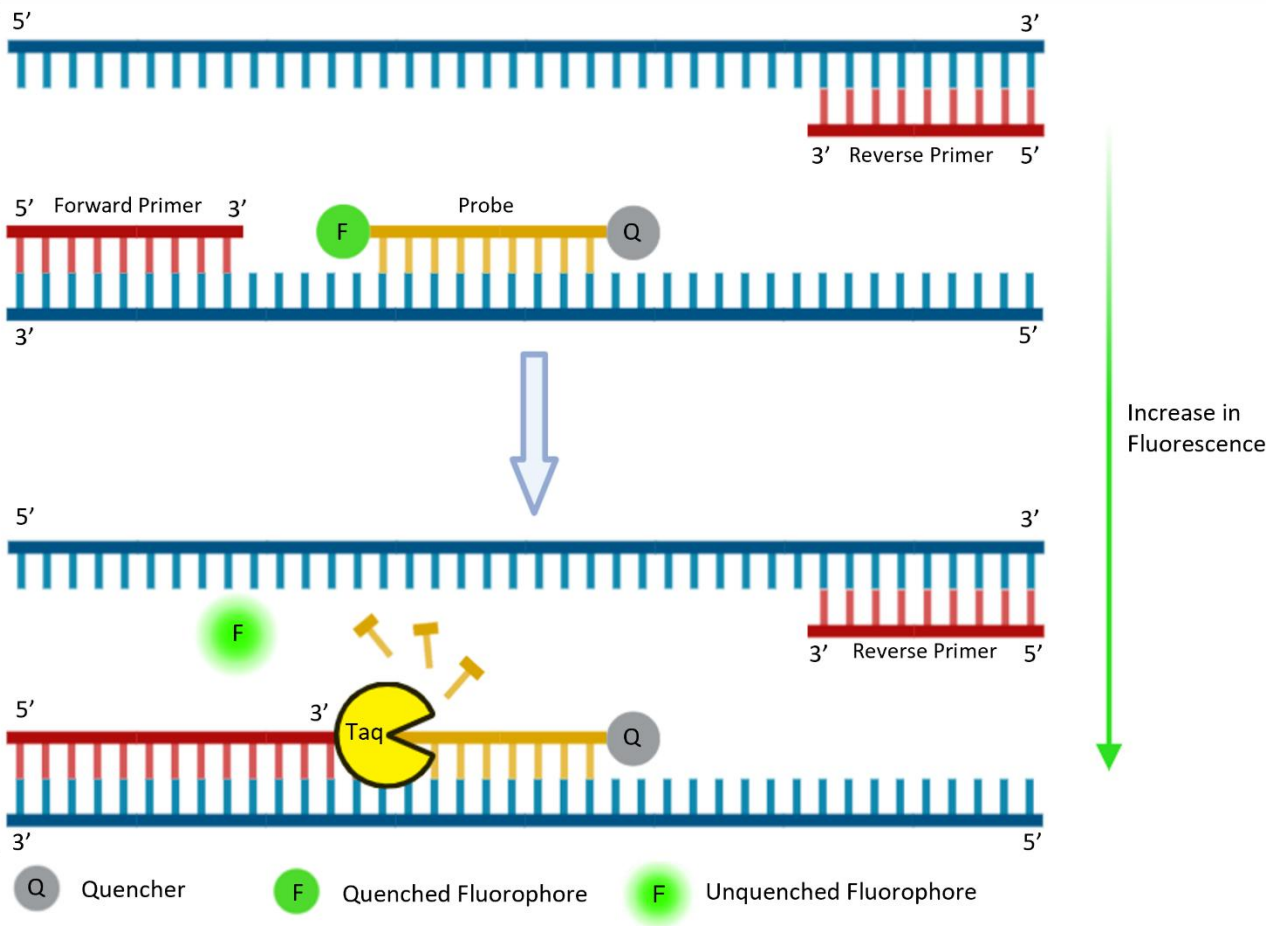


Diagram showing how dual-labelled hydrolysis probes work.

Assay Design

This qPCR assay detects a highly conserved 16S-rDNA region of Mycoplasma’s genome and can detect the presence of *Mollicutes* species most commonly associated with cell culture contamination, including, *Mycoplasma arginini*, *Mycoplasma fermentans*, *Metamycoplasma hominis*, *Mesomycoplasma hyorhinis*, *Metamycoplasma orale*, *Mycoplasmoides pneumoniae* and *Acholeplasma laidlawii*. The kit also includes an internal amplification control (IAC) for validation of the qPCR run.

Protocol

The protocol consists of three steps: nucleic acid extraction/purification, qPCR and analysis. Nucleic acids are extracted/purified from samples suspected of being contaminated with *Mollicutes* organisms. The purified nucleic acids are then amplified using MegaMix Platinum Probe qPCR Mastermix and Mycoplasma Detection Mix. Analysis of the qPCR amplification allows the user to determine if the tested sample contains *Mycoplasma*'s nucleic acids.

Nucleic Acid Extraction/Purification

This kit can be used with tradition nucleic acid extraction methods that utilise magnetic beads or columns and alternatively, it can be used with a direct-to-PCR lysis buffer such as Mycoplasma Lysis Buffer from Microzone. Mycoplasma Lysis Buffer significantly shortens the sample preparation process, with only a short heat step to release, concentrate and stabilise nucleic acids ready for PCR.

Sample preparation of cell cultures with Mycoplasma Lysis Buffer:

1. Samples should be collected when cell cultures reach 80% to 90% confluence.
2. Transfer 0.5 – 1 mL of the supernatant from the cell culture to a 1.5 mL centrifuge tube and close the lid tightly.
   Note: for suspension cell lines, stand culture flasks vertically allowing cells to settle for 30 minutes prior to removal of the supernatant.
3. Centrifuge the sample at ≥10,000 ×g for 15 min to pellet mycoplasma particles.
4. Discard the supernatant and resuspend the pellet in 100 µL Mycoplasma Lysis Buffer.
5. Vortex briefly and transfer sample to 0.2 mL PCR tubes.
6. Place in a Thermal Cycler and set profile as: 75°C for 5 mins followed by 95°C for 2 mins.
7. Centrifuge the sample for 30 s at max. speed (e.g. 10,000 × g) to pellet cellular debris.
8. Use 5 µL of the supernatant directly for qPCR or store at -20°C for later use.

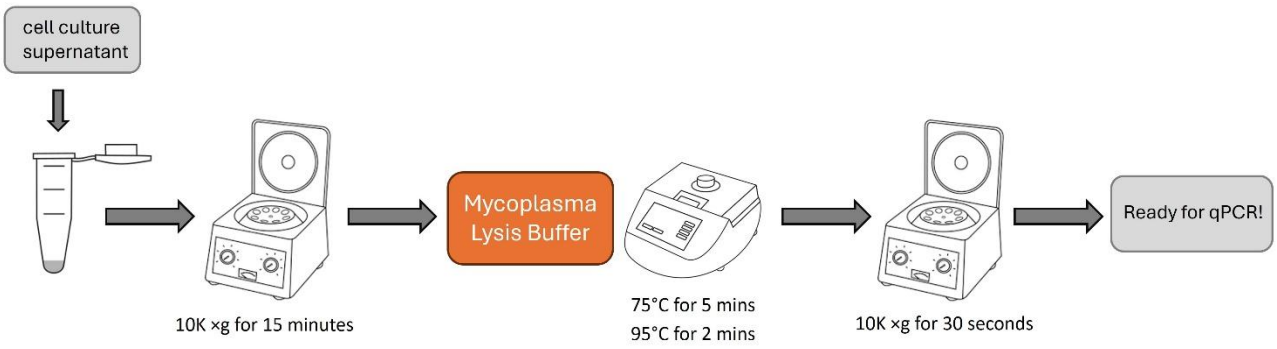


Diagram showing sample preparation with Mycoplasma Lysis Buffer workflow.

Reaction Setup

1. Thaw all reagents at room temperature.
2. Once thawed vortex all reagents and briefly centrifuge to remove any liquid from the cap.
3. Every reaction set up must allocate sufficient tubes for the number of samples, a positive, and a negative control (samples + 2 = total tubes needed). For example, 10 samples to test + 1 Positive control tube + 1 negative control tube = 12 reaction tubes required.
4. Setup should be done on ice when possible. New sterile filter pipette tips are to be used between each pipetting.
5. Generate the following mastermix, use the number of reactions tubes determined in step 3, adding 5% excess (to allow for pipetting loss) to calculate volumes:

Component	Volume
2X MegaMix Platinum qPCR Mastermix with UNG	10 µL
Mycoplasma Detection Mix	5 µL
Total volume of mastermix per sample	**15 µL**

6. Briefly vortex to mix.
7. Dispense 15 µL of mastermix into each PCR tube.
8. Add 5 µL of sample to PCR tube containing mastermix; for the positive control and negative control add 5 µL Positive Control and 5 µL Just Water, respectively.
9. Cap the tube with corresponding cap.
10. Briefly centrifuge to move all of the reaction to the base of the tube (some instruments this is not required as it is done during the PCR).
11. Transfer the tubes to the PCR instrument.

Thermal Cycling

Run the following thermal cycling profile:

Temperature	Time	Cycles	Fluorescence collection	Step
95°C	2 minutes	1		Initial Denaturation
95°C	3 seconds	40		Denaturation
60°C	20 seconds		FAM, HEX	Annealing/Extension

Interpretation of Results

Target	Reporter Fluorophore	Sample	
		Cq Value	Interpretation
Mycoplasma	FAM	<38	Positive
		≥38 or no amplification	Negative
Internal Amplification Control (IAC)	HEX	<30	Positive
		≥30 or no amplification	Negative

	Mycoplasma **(FAM)**	**IAC (HEX)**	**Reporting**
Positive Control	+	+	Valid
Negative Control	-	+	Valid
Test 1	+	+	Positive
Test 2	+	-	Positive (high concentration of *Mycoplasma* DNA)
Test 3	-	+	Negative (*Mycoplasma* Free)
Test 4	-	-	Inconclusive / PCR inhibited
Positive Control	-	+	Invalid / Retest
Negative Control	+	+	Contamination