PurMa Biologics, LLC

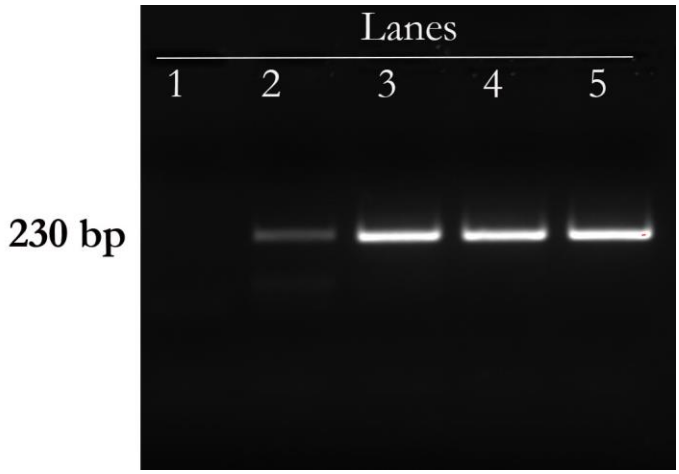
PurMa™ Mycoplasma Detection Kit

Cat #: P5M011404

User Manual

The Kit Contents (50 Reactions) Cat # P5M011404-50:

1) PurMa™ PCR 2X Mix (containing green dye and includes all the primers) (700 µl)
2) Reaction Buffer (2500µl)
3) Positive control. The lysed mycoplasma which does not carry any live mycoplasma (50ul)
4) Negative Control. Lysed COS1 cells serves as the negative control (mycoplasma free), ready to proceed to the PCR and (50µl).
5) PurMa™ Ultra-Pure Nuclease Free Water (Cat# P3W013104) (1ml)
6)



Figue1. PurMa™ Mycoplasma Detection Kit produces a 230bp fragment.

Lanes:

1. PurMa™ Negative Control.
2. PurMa™ Positive Control.
3. Contaminated medium.
4. Contaminated FBS.
5. Contaminated Cell line.

Protocol

Part 1: Tissue/Cell Homogenization

Preparing Samples

1) Take 1000 µl of cell culture supernatant including the cells. This is because the aim is to detect all the hidden/ intracellular mycoplasma as well. place them in a 1.5ml tube.
2) Centrifuge at 13000 RPM for 10 min, discard the sup and add 50µl of PurMa™ Reaction Buffer.
3) Heat the sample for exactly 5min at 95°C (to desaturate the nuclear cell wall and release the mycoplasma from heavy membrane).

Important Note:

Do not incubate for more than five min as there will be false positive result otherwise.

4) Vortex for 20 seconds
5) Transfer immediately on the ice and incubate for 5 min.
6) During the incubation on ice, vortex them two more and put back on the ice immediately.
7) Spin down briefly
8) Take 1 µl of each sample for the PCR reaction.

Part 2: PCR Reaction

PCR reaction Ingredients	µl
PurMa™ 2X PCR MIX	12.5
Sample from Part 1	1
PurMa™ Ultra-Pure Water	
Total	25

We strongly recommend preparing master mix and do not prepare tubes individually.

Part 3: Thermal Profile

Perform the PCR using the following program:

Step	Temperature (°C)	Time
Initial denaturation	94	5 min
34cycles	94	30 S
	55	45 S
	68	1 min
Final extension	68	10 min
	4	Over night

Part 4: Running the result:

Prepare a 2 % agarose and the product will show at 230 bp.

Important Note:

Due to mixing several primers, in some instances you might see a primer dimer band at 50 bp, please disregard that band as that has nothing to do with the presence/ absence of mycoplasma in your sample.