



PHOENIXDx® MYCOPLASMA MIX

FOR RESEARCH USE ONLY
2025 v 1.3

PHOENIXDx® MYCOPLASMA MIX is a fast, reliable and highly specific qPCR solution for the detection of mycoplasma contamination for example in cell culture. More than 130 different mollicute species can be detected via a specific sequence on the 16s rDNA.

PHOENIXDx® MYCOPLASMA MIX includes:

- Primers for the amplification of the 16s rDNA region
- A **PCR POSITIVE CONTROL AND A TARGET POSITIVE CONTROL**
- **FAM** Probe for the detection of mollicutes
- **HEX** Probe for the detection of the PCR Positive Control
- **dUTP** for optional UNG treatment
- **TAQ-ANTIBODY** and VitaTaq® Polymerase for High Performance and **HOTSTART** Control
- **OPTIMIZED BUFFER SYSTEM** for an efficient lysis of intact mollicute cells
- **UNIVERSAL ROX CONCENTRATION** for maximum instrument compatibility

	Cat.No.	Content
PhoenixDx® Mycoplasma Mix	PCCSKU15209	1 ml 2X Mastermix 50 µl PosCtrl

Sufficient for :

Reaction volume	Number of reactions
40 µl	50
20 µl	100
10 µl	200
5 µl	400

ADDITIONAL MATERIALS REQUIRED

- Suitable reagents / devices for DNA isolation
- Real-Time PCR Device able to detect FAM and HEX (and ROX, if required)
- Sterile filtered pipette tips
- Nuclease-free PCR tubes or plates and suitable sealing options
- Optional: Uracil-DNA glycosylase

STORAGE

Store all components at -20°C and avoid repeated freeze and thaw cycles.
Protect from light. Prepare aliquots is necessary.

GENERAL INFORMATION


- Thaw the **PHOENIXDx® MYCOPLASMA MIX** completely before use and mix gently to ensure even distribution of components
- Program your device before starting the PCR setup to allow it to reach operating temperature
- **PHOENIXDx® MYCOPLASMA MIX** uses isolated DNA as template material. It is also possible to use cell culture supernatant directly. However, as every culture is unique, a serial dilution of supernatant starting from 50% reaction volume is strongly recommended.

PCR SETUP

Optional: Perform a UNG-digestion according to the manufacturer's guidelines

➔ Program your PCR instrument.

Set **FAM** as reporter for mycoplasma detection, **HEX** for detection of the PCR Positive Control (PPC) and (if required) **ROX** for passive reference.

STEP	CYCLES	TEMPERATURE	TIME
Initial Denaturation	1	95°C	5 minutes
Amplification	50	95°C	15 seconds
		52°C 	1 minute

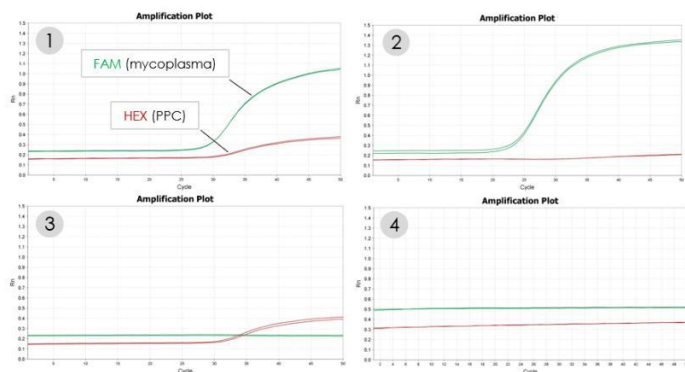
➔ Setup your PCR reactions in suitable PCR disposables:

COMPONENT	VOLUME	FINAL CONCENTRATION
PhoenixDx® Mycoplasma Mix	10 µl	1X
Template DNA/culture supernatant	X µl	1-50 ng ¹
Nuclease-free dH₂O	X µl	to 20 µl

¹ 1-50 ng of total DNA isolated from cell culture (mostly host DNA). When using culture supernatant, a serial dilution starting from 50% reaction volume is strongly recommended. For the PosCtrl supplied with the kit we recommend using 2 µl per 20 µl reaction.

➔ Load the reactions into your PCR device and start the program.

REVIEWING RESULTS



Experimental data for mycoplasma detection with PhoenixDx® Mycoplasma Mix

- 1) Mycoplasma DNA was detected, PPC was amplified, the sample is considered **positive**
- 2) A high amount of mycoplasma DNA is present in the sample and inhibits PCR Positive Control amplification, the sample is still considered **positive**
- 3) No mycoplasma DNA was detected, PPC is amplified, the sample can be considered **negative**
- 4) PCR was inhibited, the results are **inconclusive** and require reviewing.

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www.procomcure.com

or contact as via

sales@procomcure.com