PhoenixDx® Mycoplasma Mix

For research use only
2023 v 1.1

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

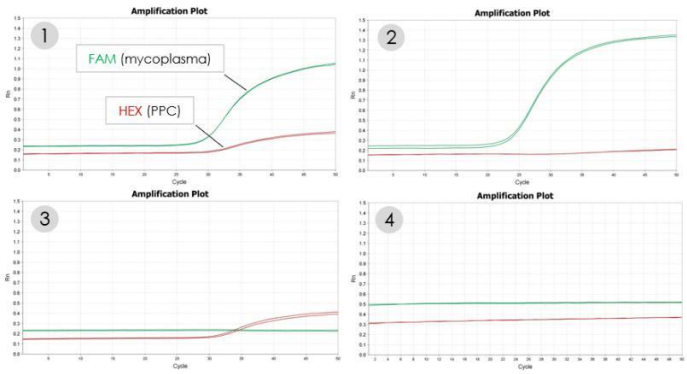
➥ Setup your PCR reactions in suitable PCR disposables:

Component	Volume	Final Concentration
PhoenixDx® Mycoplasma Mix	10 µl	1X
Template DNA/culture supernatant	X µl	1-50 ng[1]
Nuclease-free dH_2O	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 recommed using 2 µl per 20 µl reaction.

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

Reviewing Results



Experimental data fo 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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