Datasheet for ABIN6720774

PhoenixDx® Mycoplasma Mix

5 Images

Overview

Quantity:	1 mL
Application:	Quantitative real-time PCR (qPCR)

Product Details

Purpose:	MYCOPLASMA MIX is a fast, reliable and highly specific qPCR solution for the detection of mycoplasma contamination for example in cell culture.
Brand:	PhoenixDx®
Specificity:	More than 130 different mollicute species can be detected via a specific sequence on the 16s rDNA.
Cross-Reactivity (Details):	PHOENIXDX® MYCOPLASMA MIX was extensively tested for specificity against 45 non-mycoplasma DNAs. The specificity test covered viral DNA, bacterial DNA, fungal DNA and human DNA to exclude crossreactivity with the human DNA present in cell culture environment.
Characteristics:	Contamination with mycoplasma is amongst the most notorious issues associated with cell cultivation. Depending on cell type, source and culture methods, the contamination with mycoplasma, acholeplasma and ureaplasma varies between 15 and 80 % . A contamination is not only inconvenient, but also a costly issue as it often requires the elimination of precious cultures. Furthermore, the clinical use of cultured cells makes testing for mollicutes a necessity, especially for pathogens like M. pneumoniae, M. genitalium and M. hominis. PhoenixDx® offers a fast, sensitive and highly specific detection system for more than 130 Mollicute species to provide certainty when certainty is needed. Especially when working with virus samples in the cell culture, qPCR is superior to conventional detection methods as high concentrations of enveloped viruses can bias colorimetric methods like ATP conversion2. With PhoenixDx® Mycoplasma Mix, the 16s rDNA of more than

Product Details

	130 Mollicute species is targeted covering Mycoplasma, Acholeplasma and even Ureaplasma, whereas genomic, eukaryotic DNA (e.g. from the cell culture) is not amplified. To exclude false-negatives due to PCR inhibition, an additional PCR positive control is included in the mastermix. PhoenixDx® Mycoplasma Mix is not only highly specific, but also easy and fast to use due to its convenient 2X Mastermix formulation.
Components:	The PhoenixDx® Mycoplasma Mix includes: • Primers for the amplification of the 16s rDNA region • A PCR 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
Material not included:	• 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

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Comment:	PHOENIXDX® MYCOPLASMA MIX... • is MORE SENSITIVE than staining methods (detection of less than 10 genomes /reaction) • is SPECIFIC (conserved 16s rDNA target for mollicutes) • is RELIABLE (internal PCR positive control) • has LESS FALSE-POSITIVE RESULTS than enzyme-based methods • has LESS FALSE-NEGATIVE RESULTS than enzyme-based methods • requires SHORT HANDS-ON TIME and results can be obtained in less than 2 hours
Reagent Preparation:	• 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.
Assay Procedure:	Optional: Perform a UNG-digestion according to the manufacturer's guidelines

Application Details

	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. Initial Denaturation: (1 Cycle) 95 °C 5 min Amplification: (50 Cycles) 95 °C 15sec, 52 °C 1 min Setup your PCR reactions in suitable PCR disposables: PhoenixDx® Mycoplasma Mix: Volume 10 µL Final Conc. 1X Template DNA/culture supernatant: Volume X µl Final Conc. 1-50 ng Nuclease-free dH2O: Volume X µl Final Conc. to 20 µL Load the reactions into your PCR device and start the program.
Restrictions:	For Research Use only

Handling

Format:	Liquid
Handling Advice:	Protect from light. Prepare aliquots is necessary.
Storage:	-20 °C
Storage Comment:	Storage: -20 °C storageComment: Store all components at -20°C and avoid repeated freeze and thaw cycles.

Publications

Product cited in:	Johnson, Drugan, Miller, Evans: "38" in: , Vol. 1363, Issue Nucleic acids research, pp. 28-39, (1991)

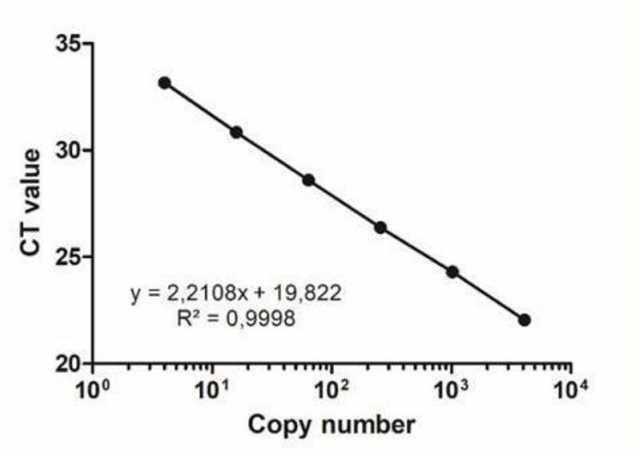


Image 1. PHOENIXDX® MYCOPLASMA MIX exhibits linearity over a broad range of target DNA input down to 8 copies of a mycoplasma genome can be detected.

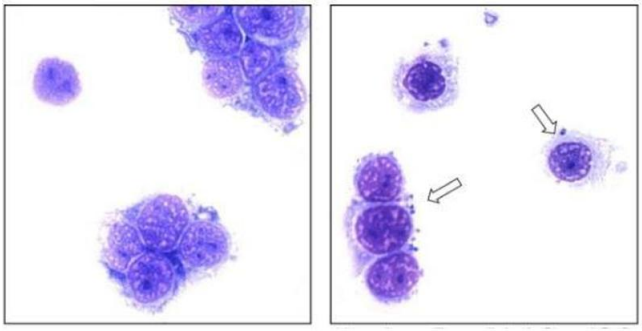


Image 2. left: Mycoplasma negative Hep-2 cells tested by PHOENIXDX® MYCOPLASMA MIX (Giemsa staining). right: Mycoplasma-like particles in PHOENIXDX® MYCOPLASMA MIX positive Hep-2 cells (Giemsa staining).

Quantitative real-time PCR

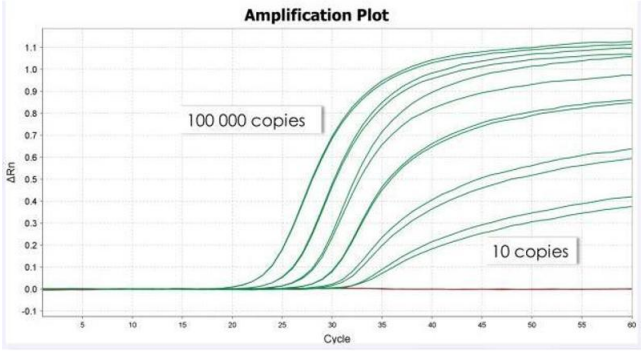
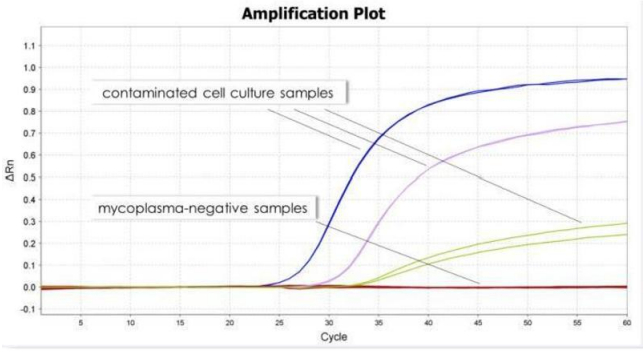
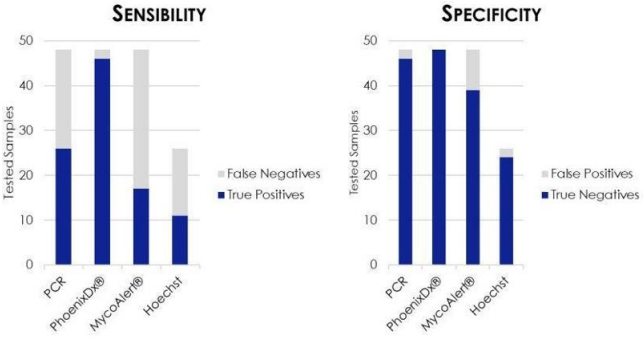


Image 3. PHOENIXDX® MYCOPLASMA MIX exhibits linearity over a broad range of target DNA input down to 8 copies of a mycoplasma genome can be detected.



Quantitative real-time PCR

Image 4. PHOENIXDX® MYCOPLASMA MIX specifically detects mycoplasma from contaminated cell culture samples (blue) and contaminated virus-propagating cell cultures (purple and yellow). Other potentially present DNAs (45 in total, human,viral, fungal and bacterial) were not detected.



Quantitative real-time PCR

Image 5. qPCR as a means of mycoplasma detection excels conventional methods like Hoechst staining in terms of sensibility and specificity.