QUALICHEK™ Mycoplasma qPCR Detection Kit

Cat No: KQDR-25 / KQDR-50 / KQDR-100

Introduction:

The QUALICHEK™ Mycoplasma qPCR Detection Kit is used to detect Mycoplasma infection of cell cultures by real-time quantitative PCR (qPCR) using Probe. The QUALICHEK™ Mycoplasma qPCR Detection Kit includes a Primer and Probe mixes. These mixes contain FAM labeled probe specific for mycoplasma species and Hex labeled probe for internal control DNA. The primer set is specific to the highly conserved the 16SrRNAcoding region in the mycoplasma genome. This allows the detection of *M. orale, M. hyorhinis, M. arginini, M. fermentans, Acholeplasma laidlawii, M. hominis*, usually encountered as contaminants in cell cultures. Furthermore, this kit can detect *M. pneumoniae, M. salivarium, M. synoviae* and *Ureaplasma* species. Eukaryotic and bacterial DNA is not amplified by QUALICHEK™ Mycoplasma Real-Time PCR Detection Kit.

The QUALICHEK™ Mycoplasma qPCR Detection Kit is capable of detecting Mycoplasma infections in cell cultures in less than three hours, depending on the spectrofluorometric thermal cycler used for detection.

Materials Provided:

Materials provided	Quantity		
	KQDR-25	KQDR-50	KQDR-100
2X qPCR Master Mix	250 ul	500 ul	1 ml
Primer and Probe Mix*	50 ul	100 ul	200 ul
Positive Control DNA	13 ul	25 ul	50 ul
50X ROX (Reference Dye); High Rox/Low Rox	13 ul	25 ul	50 ul
DNase Free Water	200 ul	400 ul	1 ml

**The internal control can be detected with a yellow filter (535–555 nm for Hex). The presence of mycoplasma DNA in the sample is indicated by an increasing fluorescence signal at 510 nm (FAM) and is usually detected with a green filter (470–510 nm).*

Storage Condition

1. Upon receipt store at -20°C
2. Avoid repeated freeze/thaw cycles
3. Aliquot the reagents, if frequent freeze and thaw is needed

Assay Procedure

1. Sample Preparation

Samples should be derived from cultures which are at 90~100% confluence. Penicillin and streptomycin in the culture media do not inhibit mycoplasma or affect test sensitivity.

To avoid false positive results, we recommend the use of the PCR grade water delivered with the kit, aerosol-preventive filter tips and gloves.

a) High Contamination

The templates for the PCR analysis are prepared by direct heating of the samples (the cell culture supernatant or the biological material).

1) 150 ul liquid supernatant of the sample is transferred into a tube
2) The supernatant is centrifuge for 5 minutes at 1,000 rpm to sediment cell debris.
3) 100 ul of the supernatant is transferred into a tube.
4) Heat the samples at 98°C for 10 min. **(Caution!! Be careful when you heat the sample at 98°C. Heating it in a PCR machine with heating lid is recommended.)**
5) The supernatant is used as template in the PCR. If the template contains PCR inhibition materials, the DNA can be purified with a commercial extraction kit.

b) Low Contamination: Enrichment of Mycoplasma by Centrifugation

1) 1.2 ml liquid supernatant of the sample is transferred into a 1.5 ml tube and centrifuge for 5 minutes at 1,500 rpm to sediment cell debris
2) 1 ml of the supernatant is transferred into a 1.5 ml tube.
3) Centrifuge for 10 minutes at 13,000 rpm to sediment mycoplasmas
4) Discard supernatant and wash the pellet once with 1 ml of PBS. Repeat step 3).
5) Discard supernatant and add 50 ul DNase free water or TE buffer to the pellet.
6) Heat the samples at 98°C for 10min, and vortex for 5~10 sec. Then, centrifuge for 5 min at 12,000 rpm with a micro centrifuge. (Caution!! Be careful when you heat the sample at 98°C. Heating it in PCR machine with heating cover is recommended.)
7) Transfer the heated supernatant to a fresh tube. This supernatant will be used as the template in the PCR
8) If the template contains PCR inhibition materials, the DNA can be purified with a commercial extraction kit.

c) Genomic DNA extraction

1) Collect 1 ml cell culture ($5x10^5$ ~ $1x10^6$ cells/ml) to a tube. Centrifuge for 10 min at 15,000 rpm.
2) DNA was isolated using a commercial kit, DNeasy® Blood and Tissue Kit (Qiagen, Valencia, CA) or equivalent products of it following the procedure provided by the vendor.
3) The concentration of genomic DNA was determined by UV260 measurement.
4) Take 1~5 ul supernatant as template for PCR reaction.

2. Preparation for qPCR

Prepare the set of reactions listed in the following table. These include two types of control reactions:
1) Positive Control reaction(s) containing mycoplasma positive control template DNA, and
2) Negative Control (no template control) reaction(s).
(Caution!! Don't vigorous vortexing.)

Reaction components	Sample reaction	Control reactions	
		Positive control	NTC (No template control)
2X qPCR Master Mix	10 ul	10 ul	10 ul
Primer & Probe mix including Internal DNA	2 ul	2 ul	2 ul
Test Sample	1~5 ul	-	-
50X High ROX*	0 ul (No ROX) or 0.4 ul (1X) High ROX or 0.4 ul (1X) Low ROX		
50X Low ROX*			
Control DNA	-	1 ul	-
DNase Free Water	Up to 20 ul		
Final volume	20 ul	20 ul	20 ul

*Instruments for ROX reference dye

Instruments	ROX
BioRad: iCycler, MyiQ, MiQ 2, iQ 5, CFX-96, CFX-384, **MJ Research:** Opticon, Option2, Chromo4, MiniOpticon **Qiagen:** Roto-Gene Q, Roto-Gene3000, Roto-Gene 6000 **Eppendorf:** Mastercycler realplex **Illumina:** Eco RealTime PCR System **Roche:** LIghtCycler 480, LightCycler 2.0	No ROX
ABI: Step-one, Step-one plus, 7000, 7300, 7700, 7900HT	High ROX
ABI: 7500, 7500 Fast, Quantstudio (3, 5, 7) **Stratagene:** MX3000, MX3005P, MX4000	Low ROX

Set up the qPCR instrument to run the PCR cycling (amplification) program specified below

Steps & Cycle			Temp (ºC)	Time
Pre-heat			95	5 min
PCR	40 Cycles	**Denature**	95	20 sec
		Anneal	60	30 sec
		Extend	72	30 sec
		Acquisition: **Mycoplasma DNA – FAM (470~510 nm), green channel** **Internal DNA – HEX (535~555 nm), yellow channel**		

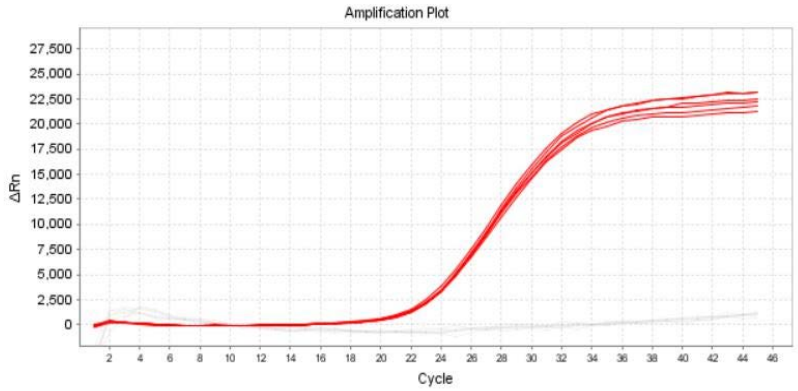
Results

A successfully performed PCR without inhibition is indicated by an increasing fluorescence signal in the internal control channel (HEX). The internal DNA can be detected with a yellow filter (535–555 nm for HEX). The presence of mycoplasma DNA in the sample is indicated by an increasing fluorescence signal at 520 nm (FAM) and is usually detected with a green filter (470–510 nm). False-negative results (due to inhibition of PCR reaction by the sample matrix) can be detected individually for each sample as these reactions do not show any fluorescence signal. Using the following table, determine whether the test cell culture is infected with Mycoplasma.

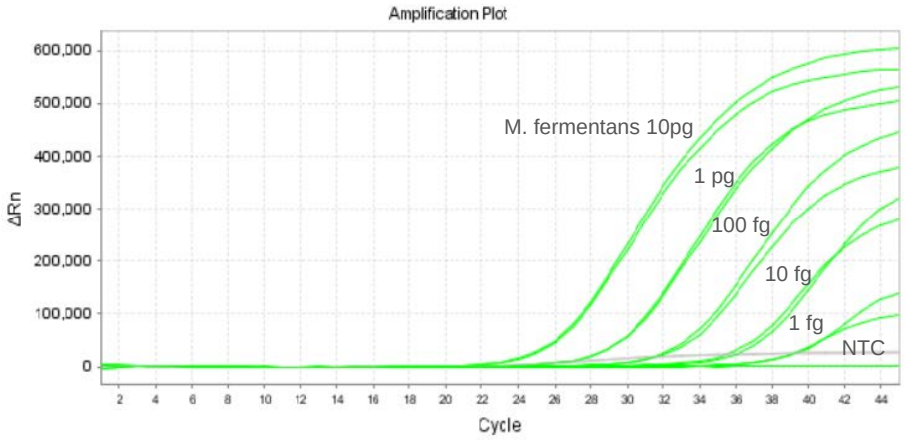
FAM channel (Mycoplasma PCR)	HEX channel (Internal DNA)	Interpretation
Positive	Positive	Mycoplasma contamination
Positive	Negative*	Mycoplasma contamination
Negative	Positive	Mycoplasma non-contamination
Negative	Negative	PCR inhibition

** In case of severe mycoplasma contamination, HEX can be not detected*

Internal Control Amplification – HEX (yellow channel)



Sample and Positive Control Amplification – FAM (green channel)



Sensitivity

The sensitivity of the PCR using this kit is 1 to 100 copies of the target DNA per reaction. Sensitivity of the assay in real culture samples depends on the quality of the sample preparation.

Specificity

The QUALICHEK™ Mycoplasma qPCR Detection Kit detect Mycoplasma species simply, reliably, and rapidly. To detect the presence of these microorganisms, the assay uses the polymerase chain reaction (PCR) to amplify a target unique to a wide variety of mycoplasmas. The kit can detect almost all kinds of *Mycoplasma species, including Acholeplasma laidlawii* and *Spiroplasma citri.* The kit does not detect other genera or cellline DNA

Please note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE"

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