BioMycoX®

Mycoplasma qPCR Detection Kit

Version:6.0

Latest update: 2024.02.01

Instruction Manual

Cat. No. QDR-25, QDR-50, QDR-100

Research Use Only. Not for Use in Diagnostic Procedures.

Contents

1. Product Information

Introduction

The BioMycoX® Mycoplasma qPCR Detection Kit is used to detect Mycoplasma infection of cell cultures by real-time PCR (qPCR) using Probe. This kit includes Primer and Probe mixes containing a FAM-labeled probe specific for mycoplasma species and a HEX-labeled probe for internal control DNA. The primer set is specific to the highly conserved the 16S rRNA coding 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 BioMycoX® Mycoplasma qPCR Detection Kit.

The BioMycoX® 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.

Kit Specificity

The BioMycoX® 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 cell-line DNA.

Kit Sensitivity

The sensitivity of the PCR using this kit is 10 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.

Prevention of carryover contamination

Take precautions to minimize the potential for carryover of nucleic acids from one experiment to the next. Use separate work areas and pipettes for template preparation and PCR setup steps.

The 2X qPCR Master Mix contains dUTP instead of dTTP. When dUTP replaces dTTP in PCR amplification, UNG treatment (Uracil-N-glycosylase, HiSense™ UDG heat labile Cat.No.SCU-100, not provided in this kit) can prevent the subsequent reamplification of dU-containing PCR products. UNG acts on single- and double-stranded dU-containing DNA by hydrolysis of uracil-glycosidic bonds at dU-containing DNA sites. With this strategy, carryover contamination is eliminated, while the template DNA (DNA containing T) remains intact.

Precaution for cross contamination

Cross contamination may occur while handling the positive control DNA contained in the kit. To prevent cross contamination, use separate locations and pipettes for handling positive control during the PCR process. Be sure to use PCR grade water included in the kit and wear gloves during experiments. Additionally, use filter tips to prevent aerosols.

2. Contents and Storage

Materials Provided

Label	Cap	QDR-25	QDR-50	QDR-100
2X qPCR Master Mix	Blue	250 µl	500 µl	1 ml
Primer and Probe Mix	Amber	50 µl	100 µl	200 µl
Positive Control DNA	Yellow	13 µl	25 µl	50 µl
50X ROX (Reference Dye) – Low ROX/High ROX	Amber	15 µl	30 µl	60 µl
DNase Free Water	White	300 µl	600 µl	1.2 ml

Storage

Upon receipt, store at -20°C.

Note

1) Repeat thawing reduces quality of product.

2) If frequent freeze and thaw is needed, aliquot the products and use in order.

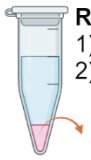
3) Check the label on the product for details.

3. Test protocol

Workflow

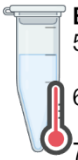
Sample Preparation

A **Boiling Method**



Remove cell debris
1) Transfer 1 ml of cell culture supernatant.
2) Centrifuge (1,000~1,500 rpm, 5min) and transfer 1ml of supernatant to a new tube.

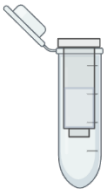
Mycoplasma havest & washing
3) Centrifuge (12,000~15,000 rpm, 10min) and discard supernatant.
4) Add 1 ml D.W or Tris-EDTA buffer and repeat previous step.



Boiling
5) Discard supernatant, add D.W 100 µl and vortexing.
6) Boiling (98℃,10 min) Centrifuge (12,000~15,000 rpm, 5 min)
7) Transfer 90 µl of supernatant to a new tube.

B **gDNA Extraction Method**

Mycoplasma harvest
1) Transfer 1 ml of cell culture supernatant.
2) Centrifuge (12,000~15,000 rpm, 10min) and discard supernatant.

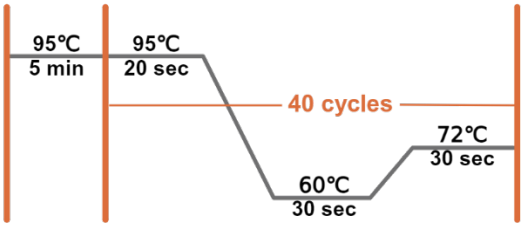


Genomic DNA extraction
3) Follow the DNA extraction kit manual.

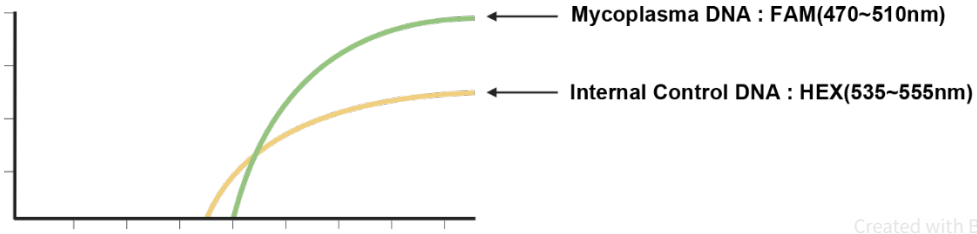
Reagent Preparation

	Sample	PC	NTC
2X qPCR Master Mix	10µl	10µl	10µl
Primer & Probe Mix	2µl	2µl	2µl
ROX dye	Depend on PCR Instruments		
Template DNA	1~5µl	-	-
Positive Control DNA	-	1µl	-
DNase Free Water	up to 20µl		

PCR Setup and Run



Analyze the results



Prepare the Template (Sample)

[A] Boiling Method

1) Transfer 1 ml of cell culture supernatant (over confluency 80%) to a 1.5 ml clean tube.
2) Centrifuge at 1,000~1,500 rpm for 5 minutes to pellet cell debris and transfer 1 ml of supernatant to a clean tube.
3) Centrifuge the tube at 12,000~15,000 rpm for 10 minutes to pellet mycoplasma and discard supernatant.
   Mycoplasma pellet may not be seen in naked eyes.
4) Add 1 ml DNase free water or Tris-EDTA buffer for washing and repeat step 3).
   This process must be performed so that the PCR reaction is not inhibited.
5) Discard supernatant completely, add DNase free water 100µl into mycoplasma pellet and mix with vortexing.
6) Heat the supernatant at 98°C for 10 minutes and centrifuge at 12,000~15,000 rpm for 5 minutes.
   Caution!! Be careful when you heat the sample at 98°C. Heating it in PCR machine with heating cover.
7) Transfer 90 µl of supernatant to a clean tube. 1~5 µl of supernatant is used as the template in PCR.

[B] Genomic DNA Extraction Method

1) Transfer 1 ml of cells with media (over confluency 80%) to a 1.5 ml clean tube.
2) Centrifuge the tube at 12,000~15,000 rpm for 10 minutes to pellet mycoplasma and discard supernatant.
3) From the next step, follow the DNA extraction kit manual you are using and use the obtained genomic DNA as a PCR template.

Prepare for qPCR

1. Prepare the set of reactions listed in the following table.

(Caution!! Don't vigorous vortexing.)

Reaction Components		Sample Reaction	Positive Control Reaction	No Template Control Reaction
Label	Cap			
2X qPCR Master Mix	Blue	10 µl	10 µl	10 µl
Primer and Probe Mix	Amber	2 µl	2 µl	2 µl
Template DNA	-	1~5 µl	-	-
Positive Control DNA	Yellow	-	1 µl	-
50X ROX (Reference Dye) *	Amber	0 µl (No ROX), Low ROX 0.4 µl (1X) High ROX 0.4 µl (1X)		
DNase Free Water	White	Up to 20 µl		
Final volume		20 µl	20 µl	20 µl

* ROX concentration for Instruments: See [Appendix A]

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

Steps & Cycle			Temp(℃)	Time
Pre-denature			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 Control DNA - HEX(535~555 nm) or VIC(526~543 nm), yellow channel**		

** Select the channel supporting in qPCR instrument.

4. Results / Trouble Shooting

A successfully performed PCR without inhibition is indicated by an increasing fluorescence signal in HEX channel for internal control. The presence of mycoplasma DNA in the sample is detected through an increasing fluorescence signal in the FAM channel.

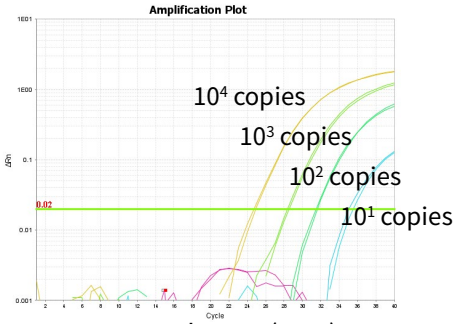
False-negative results, resulting from the inhibition of the PCR reaction by the sample matrix, can be identified individually for each sample, as these reactions do not exhibit any fluorescence signal. Using the following table, determine whether the test cell culture is infected with mycoplasma.

FAM channel (Mycoplasma PCR)	HEX or VIC channel (Internal Control DNA)	Interpretation
Positive	Positive	Mycoplasma contamination
Positive	Negative*	Mycoplasma contamination
Negative	Positive	Mycoplasma non-contamination
Negative	Negative	PCR inhibition or inadequate sample preparation

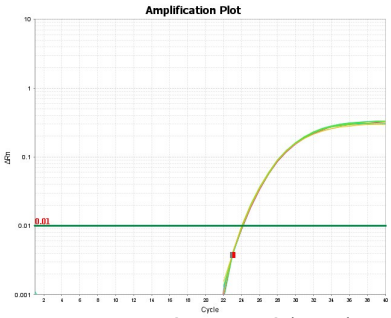
* In case of severe mycoplasma contamination, HEX can be not detected.

Amplification Result

Tested with Quantitative *M. pneumoniae* Genomic DNA (Cat. No. QPGD, CellSafe)



→ Mycoplasma (FAM)



→ Internal Control (HEX)

[Appendix A] ROX concentration for Instruments

Instruments		Reference dye
Brand	**Model**	
BioRad	iCycler, MyiQ, MiQ 2, iQ 5, CFX-96, CFX-384	No ROX
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	
ABI	5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne, StepOne plus	High ROX
ABI	7500, 7500 Fast, QuantStudio (1, 3, 5, 6, 7)	Low ROX
Stratagene	MX3000, MX3005P, MX4000	