MycoQSearch™

Mycoplasma qPCR Detection Kit (EP)

Version:6.0

Latest update: 2024.02.01

Instruction Manual

Cat. No. QDEP-100

Research Use Only. Not for Use in Diagnostic Procedures.

Contents

1. Product Information

Introduction

The MycoQSearch™ Mycoplasma qPCR Detection Kit is designed for the detection of Mycoplasma infections in cell cultures through real-time PCR (qPCR) using probes. This kit includes primer and probe mixes, with a FAM-labeled probe specific to mycoplasma species and a HEX-labeled probe for internal control DNA. The primer set is specific to the highly conserved 16S rRNA coding region in the mycoplasma genome. This allows for the detection of common contaminants in cell cultures, such as *M. orale, M. hyorhinis, M. arginini, M. fermentans, Acholeplasma laidlawii*, and *M. hominis*. Additionally, the kit can identify *M. pneumoniae, M. salivarium, M. synoviae*, and Ureaplasma species. Importantly, eukaryotic and bacterial DNA are not amplified by the MycoQSearch™ Mycoplasma qPCR Detection Kit.

The MycoQSearch™ 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 MycoQSearch™ Mycoplasma qPCR Detection Kit detects 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 1 to 10 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. Use the PCR grade water delivered with the kit, aerosol-preventive filter tips and gloves.

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.

2. Contents and Storage

Materials Provided

Label	Cap	Content
2X qPCR Master Mix	Blue	1.5 ml
Primer and Probe Mix	Amber	200 µl
Internal Amplification Control DNA	Orange	200 µl
Internal Amplification Control DNA for sample prep	Violet	2 ml
Positive Control DNA	Yellow	50 µl
50X ROX (Reference Dye) – Low ROX/high ROX	Amber	60 µl
DNase Free Water	White	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

Prepare the Template (Sample)

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

The preparation of sample for EP 2.6.7 guideline

1) Transfer 1 ml of cells with media to a 1.5 ml clean tube. (Use the number of cells according to the recommended amount of the DNA extraction kit.)

2) Centrifuge the tube at full speed (15,000 rpm) for 10 minutes to pellet mycoplasma and discard the supernatant.

3) From the next step, follow the manual for the DNA extraction kit you are using, and add 20 µl of Internal Amplification Control DNA for sample prep (Violet cap) at the step of adding Proteinase K.

4) In the elution step, 50 µl of the elution volume is obtained, and the genomic DNA is used 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		gDNA Sample Reaction	Positive Control Reaction	No Template Control Reaction
Label	Cap			
2X qPCR Master Mix	Blue	15 µl	15 µl	15 µl
Primer and Probe Mix	Amber	2 µl	2 µl	2 µl
Template DNA	-	5 µl	-	-
Internal Amplification Control DNA	Orange	- *	2 µl	2 µl
Positive Control DNA	Yellow	-	1 µl	-
50X ROX (Reference Dye) **	Amber	0 µl (No ROX) Low ROX 0.6 µl (1X) High ROX 0.6 µl (1X)		
DNase Free Water	White	Up to 30 µl		
Final volume		30 µl	30 µl	30 µl

* In the sample reaction, internal amplification control DNA is not added separately because the sample already include internal amplification control DNA through the sample preparation of DNA extraction procedure.

** ROX concentration for Instruments: See [Appendix B]

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	45 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. Trouble Shooting / Results

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.

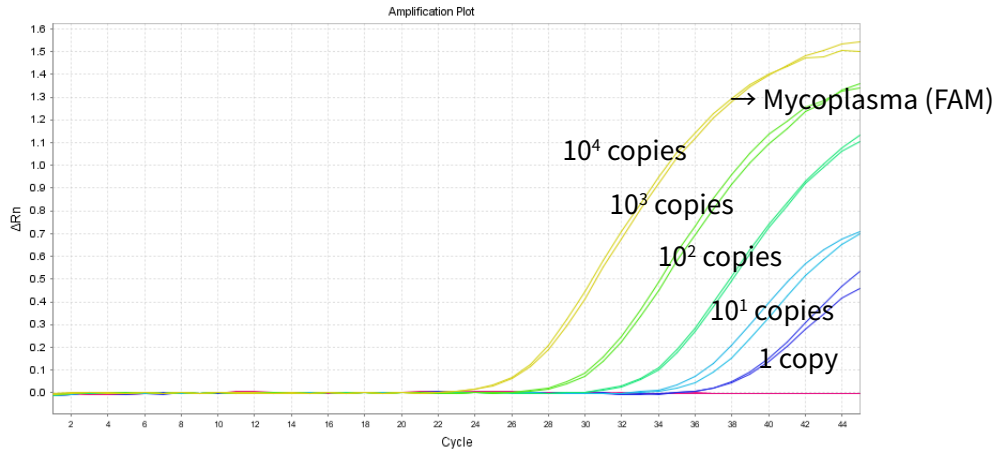
False-negative results, resulting from the inhibition of the PCR reaction by the sample matrix, can be identified individually for each sample, as these reactions do not exhibit any fluorescence signal. Using the following table, determine whether the test cell culture is infected with mycoplasma.

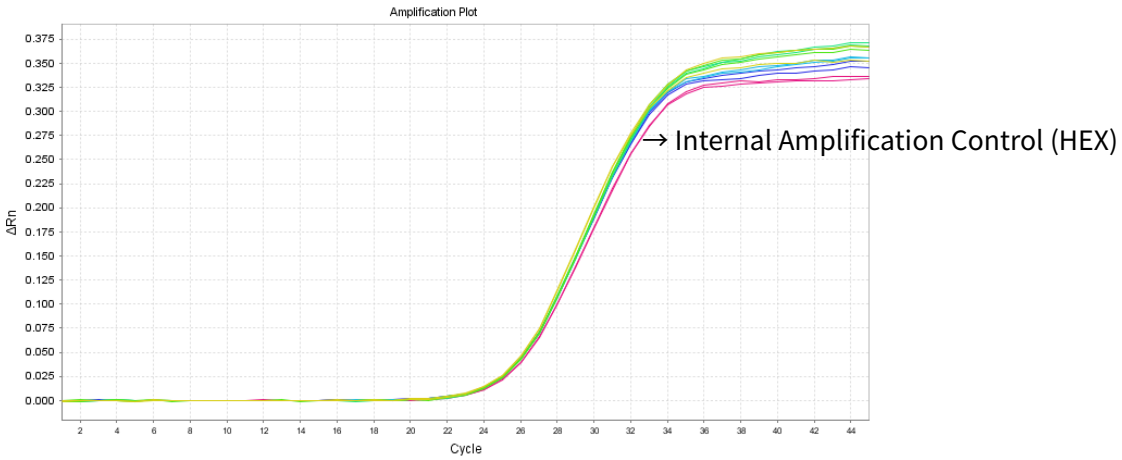
FAM channel (Mycoplasma PCR)	Cy5 channel (Positive control DNA)	HEX or VIC channel (Internal Control DNA)	Interpretation
Positive (Ct value < 40)	Positive	Positive	Positive control DNA contamination
Positive (Ct value < 40)	Positive	Negative*	Positive control DNA contamination
Negative	Negative	Positive	Mycoplasma non-contamination
Negative	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. orale* Genomic DNA (Cat. No. QOGD, CellSafe)





[Appendix A] Genomic DNA Extraction

QIAamp® UCP Pathogen Mini Kit (Cat# 50214, Qiagen)

Preparation

Things to do before starting

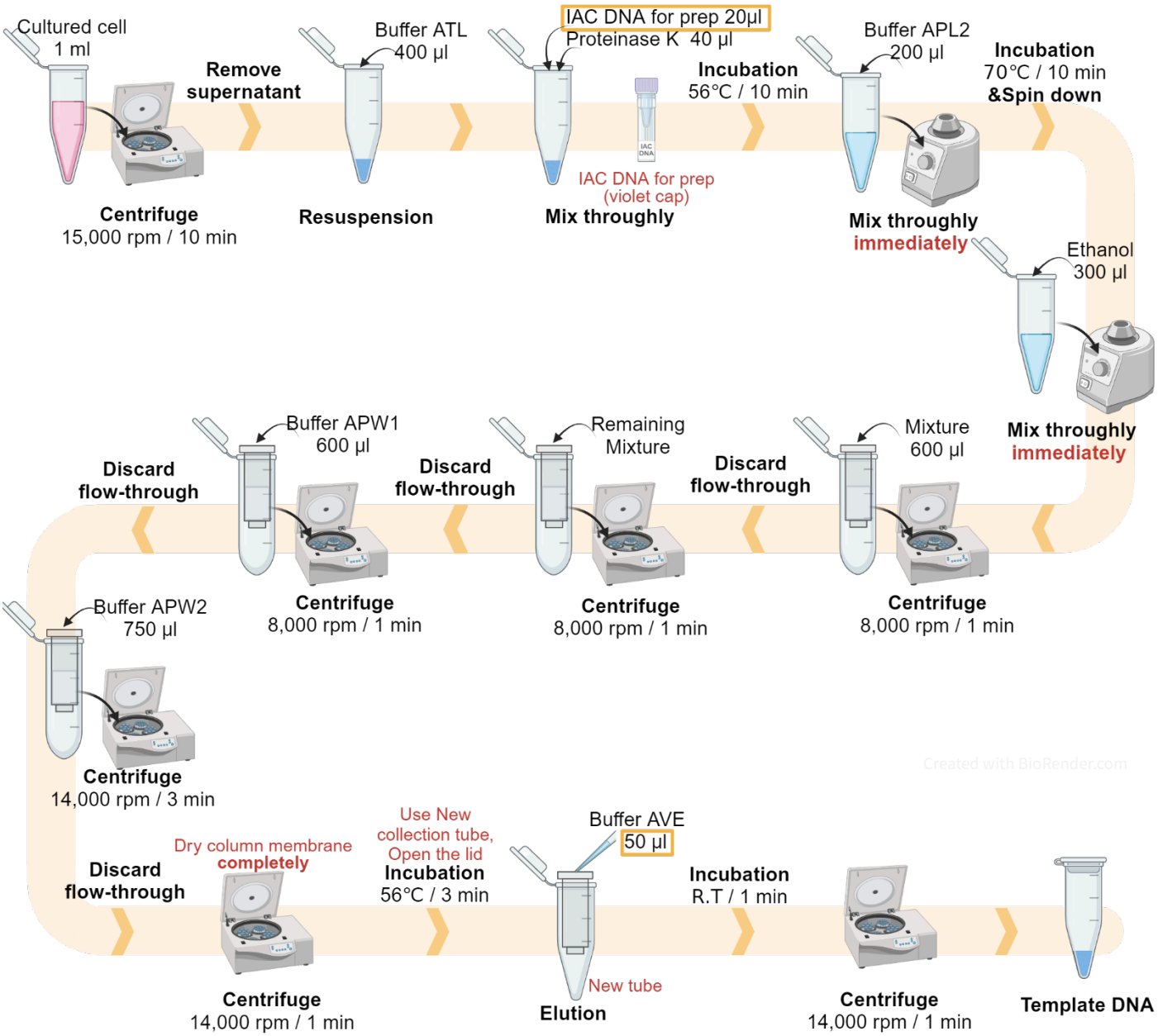
Buffer ATL or Buffer APL2 may form a precipitate upon storage. If necessary, warm to 56°C until the precipitate has fully dissolved.

Preheat a thermomixer, shaking water bath, or rocking platform to 56°C for use in step 3) and 70°C for use in step 5).

Buffer APW1 and Buffer APW2 are supplied as concentrates. Before using for the first time, add the appropriate amount of ethanol (96~100%) as indicated on the bottle to obtain a working solution.

Equilibrate Buffer AVE to room temperature for elution.

Procedure



1) Collect 1 ml cell culture to a tube. Centrifuge for 10 min at maximum speed (15,000 rpm).

When using a frozen cell pellet, allow cells to thaw before centrifuging until the pellet can be dislodged by gently flicking the tube.

2) Discard the supernatant, tap the pellet to fully resuspend it, and then resuspend it in 400 µl of Buffer ATL.

3) Add 40 µl proteinase K and 20 µl Internal Control DNA*. Mix thoroughly by tapping and incubate at 56°C for 10 min. Continue with step 4).

*The Internal Amplification Control DNA for sample prep of the MycoQSearch™ kit is used to verify the DNA extraction step as well.

4) Add 200 µl Buffer APL2.

It is essential that the sample and Buffer APL2 are mixed immediately and thoroughly by vortexing to yield a homogeneous solution.

5) Incubate at 70°C for 10 min. Then briefly spin the tube to remove drops from the inside of the lid.

6) Add 300µl ethanol (96~100%) to the sample and mix thoroughly by vortexing.

It is important that the sample and the ethanol are mixed thoroughly to yield a homogeneous solution.

7) Carefully apply 600 µl of the mixture from step 6) into the QIAamp UCP Mini spin column placed in a 2 ml collection tube. Centrifuge at 6,000 x g (8,000 rpm) for 1 min. Discard flow-through and collection tube.

8) Repeat step 7) by applying the remaining mixture from step 6) to the QIAamp UCP Mini spin column

9) Place the QIAamp UCP Mini spin column in a new 2 ml collection tube (not provided), add 600 µl Buffer APW1, and centrifuge for 1 min 6,000 x g (8,000 rpm). Discard flow-through and collection tube.

10) Place the QIAamp UCP Mini spin column in a new 2 ml collection tube (not provided), add 750 µl Buffer APW2, and centrifuge for 3 min at full speed (20,000 x g; 14,000 rpm).

11) Remove the 2 ml collection tube solution and centrifuge at full speed (20,000 x g; 14,000 rpm) for 1 minutes to dry the column membrane.

It is important to dry the membrane of the QIAamp UCP Mini spin column since residual ethanol may interfere with subsequent reactions.

Following the centrifugation step, remove the QIAamp UCP Mini spin column carefully so that the column does not come into contact with the flow-through, since this will result in carryover of ethanol. If carryover of ethanol occurs, empty the collection tube, then reuse it in another centrifugation.

12) Place the QIAamp UCP Mini Column into a new 2 ml collection tube. Open the lid and incubate the assembly at 56°C for 3 min to dry membrane completely.

13) Place the QIAamp UCP Mini spin column in a clean 1.5 ml elution tube, and pipet 50 µl Buffer AVE directly onto the QIAamp UCP Mini membrane. Incubate at room temperature for 1 min.

14) Centrifuge for 1 min at full speed (20,000 x g; 14,000 rpm) to elute.

[Appendix B] 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	