BioMycoX®

Mycoplasma PCR Detection Kit

Version:6.0

Latest update: 2024.02.01

Instruction Manual

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

Research Use Only. Not for Use in Diagnostic Procedures.

Contents

1. Product Information

Introduction

BioMycoX® Mycoplasma PCR Detection kit utilizes the polymerase chain reaction (PCR), which is the method of choice for highest sensitivity in the detection of Mycoplasma contamination in cell cultures and other cell culture derived biologicals. 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 PCR Detection kit.

Characteristics

• Detecting almost all kinds of mycoplasma species.

• Detecting the presence of contaminated mycoplasma in cell lines and cell culture media.

• Providing validity of test results by internal control.

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 PCR 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	D-25	D-50	D-100
2X PCR Master Mix	Blue	250 µl	500 µl	1 ml
Primer Mix	Red	50 µl	100 µl	200 µl
Positive Control DNA	Yellow	13 µl	25 µl	50 µl
DNase Free Water	White	150 µl	300 µl	600 µl

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.

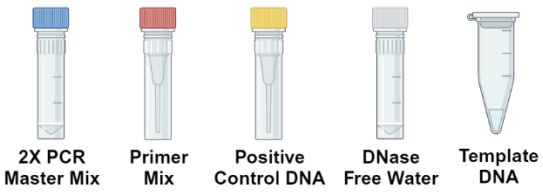
Boiling
5) Discard supernatant, add D.W 100 µl and vortexing.
6) Boiling (98°C,10 min) Centrifuge (12,000~15,000 rpm, 5 min)
7) Transfer 90 µl of supernatant to a new tube.

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.

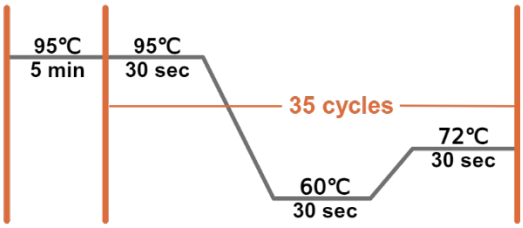
Genomic DNA extraction
3) Follow the DNA extraction kit manual.

Reagent Preparation



	Sample	PC	NTC
2X PCR Master Mix	10µl	10µl	10µl
Primer Mix	2µl	2µl	2µl
Positive Control DNA	-	1µl	-
Template DNA	1~5µl	-	-
DNase Free Water	up to 20µl		

PCR Setup and Run



Analyze the results

1. Infected cell
2. No Template Control
3. Positive Control

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 100 µl 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 PCR

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 PCR Master Mix	Blue	10 µl	10 µl	10 µl
Primer Mix	Red	2 µl	2 µl	2 µl
Template DNA	-	1~5 µl	-	-
Positive Control DNA	Yellow	-	1 µl	-
DNase Free Water	White	Up to 20 µl		
Final volume		20 µl	20 µl	20 µl

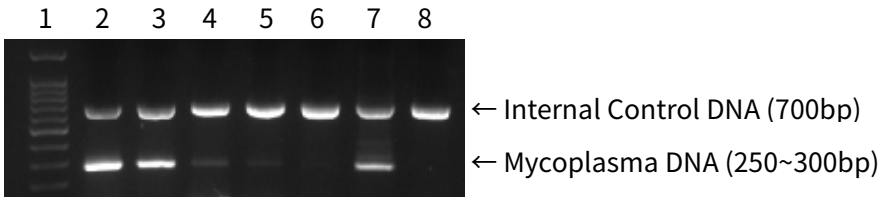
2. Set up the qPCR instrument to run the PCR cycling (amplification) program specified below.

Steps & Cycle			Temp(℃)	Time
Pre-denature			95	5 min
PCR	35 Cycles	Denature	95	30 sec
		Anneal	60	30 sec
		Extend	72	30 sec

4. Results / Trouble Shooting

Gel Electrophoresis Result

Tested with Quantitative *M. arginini* Genomic DNA (Cat. No. QAGD, CellSafe)



1. 100bp ladder
2. 10^4 copies
3. 10^3 copies
4. 10^2 copies
5. 10^1 copies
6. 1 copy
7. Positive Control
8. No Template Control

When mycoplasma contamination exists, a band with around 250-300bp appears. An internal control DNA band with around 700bp means the right performance of PCR reaction.

1) Recommend performing no template control reaction and positive control reaction by adding 1µl of positive control DNA.
2) If the PCR reaction is inhibited by high FBS concentration, the use of genomic DNA as a template may be helpful.
3) PCR inhibiting substances may accumulate in the medium of cells (e.g., Hybridoma cell, etc.). In this case, the use of diluted sample or genomic DNA as a template may be helpful.
4) In case of severe mycoplasma contamination, internal control DNA band can be not appeared.