BioMycoX®
Mycoplasma qPCR Detection Kit

Instruction Manual

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

Research Use Only. Not for Use in Diagnostic Procedures.

Introduction

The *BioMycoX®* Mycoplasma qPCR Detection Kit is used to detect *Mycoplasma* infection of cell cultures by real-time quantitative PCR (qPCR) using Probe. The *BioMycoX®* 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 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* Real-Time PCR 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 more than 50 different *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 100 copies of the target DNA per reaction. Sensitivity of the assay in real culture samples depends on the quality of the sample preparation.

Materials Provided

Materials Provided	Quantity			
	QDR-Sample	QDR-25	QDR-50	QDR-100
2X qPCR Master Mix (Blue Cap)	100µl	250µl	500µl	1ml
Primer and Probe Mix* (Amber Tube and Cap)	20µl	50µl	100µl	200µl
Positive Control DNA (Yellow Cap)	5µl	13µl	25µl	50µl
50X ROX (Reference Dye); High Rox/Low Rox (Amber Tube and Cap)	5µl	13µl	25µl	50µl
DNase Free Water	80µl	200µl	400µl	1ml

*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 Conditions

Upon receipt, store at -20℃. Once thawed, full activity is guaranteed for 6 months. Repeat thawing reduces quality of product.

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 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.

1. **High contamination: Heat-inactivation of the sample material**
   The templates for the PCR analysis are prepared by direct heating of the samples (the cell culture supernatant or the biological material).
   1) 150µl liquid supernatant of the sample is transferred into a sterile reaction tube.
   2) The supernatant is centrifuged (5 minutes, 1,000rpm) to sediment cell debris.
   3) 100µl of the supernatant is transferred into a sterile reaction tube.
   4) The supernatant is incubated at 98℃ for 10 minutes
   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.

2. **Low contamination: Enrichment of mycoplasma by centrifugation**
   1) 1.2ml liquid supernatant of the sample is transferred into a 1.5ml tube and centrifuged (5 minutes, 1,000 rpm) to sediment cell debris.
   2) 1ml of the supernatant is transferred into a 1.5ml tube.
   3) Centrifuged (10 minutes, 13,000 rpm) to sediment mycoplasma particles.
   4) Discard supernatant and wash the pellet once with 1ml of PBS. Repeat step 3).
   5) Discard supernatant and add 50µl DNase free water or TE buffer to the pellet.
   6) Heat the samples at 98℃ for 10min, and vortex for 5~10 sec. Then, centrifuge for 5 min at 12,000 rpm with a microcentrifuge. (*Caution!! Be careful when you heat the sample at 98℃ 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.

3. **Genomic DNA extraction**
   1) Prepare $5x10^5$ ~ $1x\ 10^6$ cells
   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 1~5µl supernatant as template for PCR reaction.

Prepare for qPCR

1. 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).

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

***Instruments for ROX reference dye**

Instrument	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

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

Steps & Cycle			**Temp(℃)**	**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(470~510nm), Green Channel** **Internal DNA - HEX(535~555),Yellow Channel**		

Results

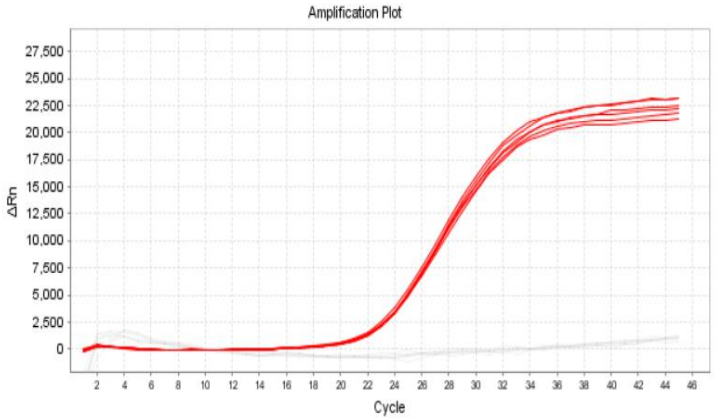
A successfully performed PCR without inhibition is indicated by an increasing fluorescence signal in the internal control channel (HEX). The internal DNA can be detected with a yellow filter (535–555 nm for HEX). The presence of mycoplasmal DNA in the sample is indicated by an increasing fluorescence 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.

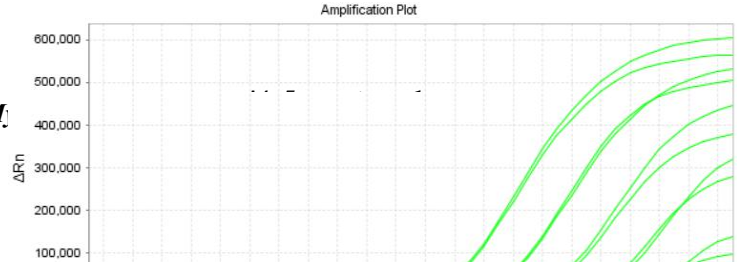
Using the following table, determine whether the test cell culture is infected with *Mycoplasma*.

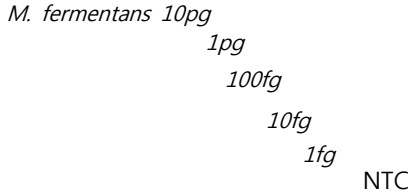
FAM Channel (Mycoplasma PCR)	*HEX Channel (Internal DNA)*	*Interpretation*
Positive	*Positive*	*Mycoplasma contamination*
Negative	*Positive*	*Mycoplasma non-contamination*
Negative	*Negative*	*PCR inhibition*

Internal Control Amplification – HEX (Yellow channel)



Sample and Positive Control Amplification – FAM (Green channel)





Mycoplasma species detected by *BioMycoX* Mycoplasma qPCR Detection Kit

1. *Acholeplasma laidlawii*
2. *Mycoplasma arginini*
3. *Mycoplasma fermentans*
4. *Mycoplasma hyorhinis*
5. *Mycoplasma orale*
6. *Mycoplasma salivarium*
7. *Mycoplasma agalactica*
8. *Mycoplasma alligatoris*
9. *Mycoplasma anatis*
10. *Mycoplasma arthritidis*
11. *Mycoplasma bovis*
12. *Mycoplasma bovigenitalium*
13. *Mycoplasma capricolum*
14. *Mycoplasma cloacale*
15. *Mycoplasma falconis*
16. *Mycoplasma faucium*
17. *Mycoplasma flocculare*
18. *Mycoplasma gallinarum*
19. *Mycoplasma gallisepticum*
20. *Mycoplasma genitalium*
21. *Mycoplasma hominis*
22. *Mycoplasma hyopneumoniae*
23. *Mycoplasma hyopharyngis*
24. *Mycoplasma hyorhinis*
25. *Mycoplasma hyosynoviae*
26. *Mycoplasma imitans*
27. *Mycoplasma maculosum*
28. *Mycoplasma meleagridis*
29. *Mycoplasma mycoides*
30. *Mycoplasma muris*
31. *Mycoplasma neurolyticum*
32. *Mycoplasma opalescens*
33. *Mycoplasma penetrans*
34. *Mycoplasma pirum*
35. *Mycoplasma pneumonia*
36. *Mycoplasma primatum*
37. *Mycoplasma pulmonis*
38. *Mycoplasma putrefaciens*
39. *Mycoplasma salivarium strain*
40. *Mycoplasma spermatophilum*
41. *Mycoplasma sp. ovine/caprine*
42. *Mycoplasma sp. putative*
43. *Mycoplasma strain*
44. *Mycoplasma suis*
45. *Mycoplasma sualvi*
46. *Mycoplasma synoviae*
47. *Mycoplasma timone*
48. *Spiroplasma citiri*
49. *Ureaplasma diversum*
50. *Ureaplasma urealyticum*