BioMycoX® Mycoplasma PCR Detection Kit

Cat. No. D-Sample, D-25, D-50 and D-100
Storage Temperature -20℃

BioMycoX® Mycoplasma 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 Detection kit.

1. Characteristics

- Detecting almost all kinds of mycoplasma species.
- Providing validity of test results by internal control.
- Ready-to-use, optimized PCR premix type.

2. Kit Contents

Matreial Provided	Quantity			
	D-sample	D-25	D-50	D-100
2xPCR Premix (Blue Cap)	50µl	250µl	500µl	1ml
Primer Mix (Red Cap)	10µl	50µl	100µl	200µl
Positive Control DNA (Yellow Cap)	5µl	13µl	25µl	50µl
DNase Free Water (White Cap)	50µl	150µl	300µl	600µl

3. Storage/Stability Conditions

Upon receipt, store at -20℃.

Note:
1) Repeat thawing reduces quality of product.
2) If frequent freeze and thaw is needed, aliquot the products and use in order

4. Expiration Date: 12 months

Note: Please check the label on the product for details.

5. Mycoplasma Detection Protocols

I. Preparation of Sample (Template)

1) Thaw the kit components at room temperature. Spin them briefly in a microcentrifuge to collect the material in the bottom of the tube.
2) Transfer 1.2ml of cell culture supernatant to a microcentrifuge tube.
3) Spin at 1,000rpm for 5minutes to pellet cellular debris.
4) Transfer 1ml of supernatant to a fresh tube.
5) Centrifuge the tube at 13,000rpm for 10 minutes to pellet mycoplasma.
6) Discard supernatant and wash the pellet once with 1ml of PBS. Repeat step 5).
7) Discard supernatant and add 50µl DNase free water or TE buffer to the pellet.
8) 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.*)
9) Transfer the heated supernatant to a fresh tube. This supernatant will be used as the template in the PCR.

II. PCR reaction

10) Prepare the set of reactions listed in the following table. (*Caution!! Don`t vigorous vortexing.*)

Reaction components	Sample Reaction	Positive Reaction	Negative Reaction
2XPCR Premix	10µl	10µl	10µl
Primer mix	2µl	2µl	2µl
Sample	3~5µl	-	-
Positive control DNA	-	1µl	-
DNase Free Water	Up to 20µl		
Final Volume	20µl	20µl	20µl

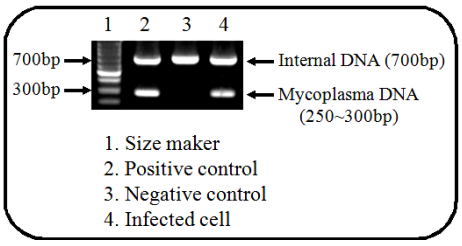
11) Perform PCR reaction as in the following:

Temperature	Time	Cycle
95℃	5 min	1 cycle
95℃	30 sec	35 cycles
55℃	30 sec	
72℃	30 sec	

12) Apply 5~10µl each of PCR products to the gel electrophoresis.

III. Result

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



Note:
1) Recommend to perform one negative control without sample and one positive control reaction by adding 1µl of mycoplasma 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 hybridoma cell. In this case, the use of diluted sample or genomic DNA as a template may be helpful.

6. Related Products

BioMycoX® Mycoplasma Elimination Kit
Cat. No. E-01/02/03 (Light /Medium/Heavy contamination)
BioMycoX® Mycoplasma Prevention Spray
Cat. No. P-1000 (1L)
CryoBanker® Cell Freezing Medium, Serum Free
Cat. No. CB-50/100 (50ml/100ml)