AllDetect PCR Mycoplasma Detection Kit

For quick detection of mycoplasma contamination

Cat. No: ABTGDT311-01

Storage: at -20℃ for two years.

Description

AllDetect PCR Mycoplasma Detection Kit is designed to detect the presence of mycoplasma contamination by PCR in biological materials, such as cell cultures or cell culture related materials. Highly specific primers have been designed to amplify a fragment of 16S rRNA coding DNA that is conserved across all commonly-known mycoplasma species. The kit includes an optimized supermix and primer, ultrapure water and positive control template. Using this kit, cell culture supernatants can be tested directly without DNA extraction. The kit provides a very easy to use, simple, fast (within 2 hours), specific and sensitive PCR-based mycoplasma detection method.

Highlights

- High Sensitivity-Able to detect as low as 20 copies of mycoplasma genome.
- High Specificity-Only detect mycoplasma DNA, not eukaryotic and bacterial DNA.
- Simple and Easy to Use-Ready-to-use, optimized master mix and no need for DNA extraction.
- Positive and Negative Control-Ensure reliability and accuracy of the results.

Kit Contents

Component	ABTGDT311-01 (100 rxns)
AllDetect PCR Myco SuperMix (2×)	1 ml
Myco Primer Mix	40 µl
Myco Positive Control Template	40 µl
MycoFree Water	1 ml

Procedures

- Preparation of samples

  a) Adherent cell

  Transfer 40 µl of the cell culture supernatant (cell should be cultured for 2-3 days with about 80% confluence) to a PCR tube, incubate at 95℃ for 10 minutes in a thermo cycler, then use it as the test template for the following PCR.

  b) Suspension cell

  Transfer the cell culture (cell should be cultured for 2-3 days with a density of about 106 cells per milliliter) to a tube and centrifuge at 2000×g for 1 minute, then transfer 40 µl of the cell supernatant to a PCR tube, incubate at 95℃ for 10 minutes in a thermo cycler, then use it as the test template for the following PCR.

  c) Serum

  Make a 20-fold dilution of the serum. Then transfer 40 µl to a PCR tube, incubate at 95℃ for 10 minutes in a thermo cycler, and then use it as the test template for the following PCR.

  Note: Test samples can be saved at -20℃ for more than 1 month before or after heat treatment. For cell that has been cultured for longer than 4 days, make a 10-fold dilution before test.

- PCR setup

  To avoid mycoplasma contamination, set up the following PCR reactions at a designated PCR area (MycoFree Water as negative control and Myco Positive Control Template as positive control). Keep everything on ice during the set up.

Component	Volume
Template	2 µl
Myco Primer Mix	0.4 µl
AllDetect Myco PCR SuperMix（2×）	10 µl
MycoFree Water	7.6 µl
Total volume	20 µl

• PCR amplification

Program the thermocycler according to the program below:

94°C	4 min	
94°C	30 sec	35 cycles
60°C	30 sec	
72°C	30 sec	
72°C	5 min	

• Agarose Gel electrophoresis

Load 10 µl of the PCR product on 1.5% agarose gel. Resolve by electrophoresis.

• Interpretation of the result

Analyze and confirm the results of mycoplasma contamination by comparing with the positive and negative control. The size of positive band is about 350 bp.

Notes

• Ensure the AllDetect Myco PCR SuperMix (2×) and other compounds are fully thawed, mixed evenly before use.
• Antibiotics such as penicillin, streptomycin or serum in the cell culture samples will not affect the detection results of this kit.
• In order to obtain the best detection result, cell should be cultured for 2-3 days with about 80% confluence (adherent cells) or the cell density is about 106 per milliliter (suspension cell).
• Throughout the process, please strictly follow the PCR operating standards in a designated area to avoid cross contamination.
• When setting PCR reactions, please wear mask, since oral cavity contains mycoplasma, which may contaminate the sample and cause false positive.
• In order to ensure the reliability and accuracy of the results and meet the publishing requirements of major journals, we recommend that cell cultures and cell culture reagents be regularly tested for mycoplasma contamination. Cell culture is very easy to be contaminated by mycoplasma, so it is recommended to use the AllSafe Mycoplasma Prevention Reagent (Cat. No: ABTGFT501) which has much better effects against mycoplasma to replace the Penicillin & Streptomycin. If Mycoplasma contaminated cells are precious or difficult to culture, it is recommended to use the AllSafe Mycoplasma Elimination Reagent (Cat. No: ABTGFT401, ABTGFT411) to eliminate the mycoplasma and rescue the cells.