

e-Myco™ plus Mycoplasma PCR Detection Kit

RUO Research Use only

REF 25237 Σ 48

REF 25238 Σ 8

-22°C -18°C

Test for the detection of Mycoplasmas by PCR analysis

This kit is covered by patents owned by Abbott Molecular Inc. (US Pat. No. 5,851,767 and its foreign counterparts)

BACKGROUND INFORMATION

Mycoplasma is a genus of bacteria which lack a cell wall. Without a cell wall, they are unaffected by many common antibiotics such as penicillin or other beta-lactam antibiotics that target cell wall synthesis. They can be parasitic or saprotrophic. Several species are pathogenic in humans, including *M. pneumoniae* and *M. genitalium*. Mycoplasma species are often found in research laboratories as contaminants in cell culture. Mycoplasma cell culture contamination occurs due to contamination from individuals or contaminated cell culture medium ingredients.

e-Myco™ plus Mycoplasma PCR Detection Kit greatly simplifies testing and detection of mycoplasma contamination in cell cultures. With PCR testing, reliable results are obtained within a few hours, since the presence of contaminant mycoplasmas can be easily and sensitively detected by simply verifying the bands of amplified DNA fragments after gel electrophoresis. The e-Myco™ plus Mycoplasma PCR Detection has been shown to be a highly sensitive, specific and rapid method for the detection of mycoplasmas contamination in cell cultures.

Though the gene sequences for 16S rRNA are very similar in most Mycoplasma species, there are some differences in the sequences of 16S rRNA gene between certain Mycoplasma species and the other species.

Specific primers set of e-Myco™ plus Mycoplasma PCR Detection Kit were designed from DNA sequences that are coding for highly conserved 16S rRNA with considering above point. Thus e-Myco™ plus Mycoplasma PCR Detection Kit can be used in the detection of a more broad range of Mycoplasma species, compared with any other commercially available PCR-based Mycoplasma detection kit, without interfering with animal or bacterial DNA.

An exogenous internal control of this product was constructed to identify false negative results in each reaction. The internal control was designed in such a way that the primers set was used to amplify the internal control and target DNA, which were differentiated by size. Furthermore, the sample control was provided with this kit for using in verifying the effectiveness of template DNA. So, You may easily check your sample preparation. In addition, the use of 8-methoxypropalen (8-MOP) was adopted in this kit. 8-MOP is helpful to prevent cross-contamination by PCR products from earlier experiments.

CHARACTERISTICS

- Premix Type** : This e-Myco™ plus Mycoplasma PCR Detection Kit contains all the components for the PCR reaction. You just add a template and DW.
- Wide Range of Detectable Mycoplasmas** : You can detect not only five common cell culture-infecting species of mycoplasma but also other various species of mycoplasma over 8 genus 209 species (refer to Technical Guide).
- Exogenous Internal Control** : Internal control embedded in the product prevents misjudgment that possibly arises from an erroneous PCR test.
- Sample Control** : You can verify easily the effectiveness of template gDNA by checking the amplification from sample control.
- Species Determination** : You can determine the species of mycoplasma by sequencing the amplified PCR products.
- Elimination of Cross-Contamination** : 8-MOP prevents cross-contamination by PCR products.

INTENDED USE

- For Research Use Only, Not for use in diagnostic procedures.

e-Myco™ plus Mycoplasma PCR Detection Kit is developed, designed, and sold for research purpose only. It is not intended to be used for human or animal diagnosis of diseases. Do not use internally or externally in humans or animals. Prior to using it for other purposes, the user must validate the system in compliance with the applicable law, directives, and regulations.

REQUIREMENTS INSTRUMENT

- Pipettes and pipette tips (aerosol barrier)
- G-spin™ Total DNA Extraction Mini Kit
- Thermal cycler
- Vortex mixer
- Disposable gloves
- Heat block

DESCRIPTION

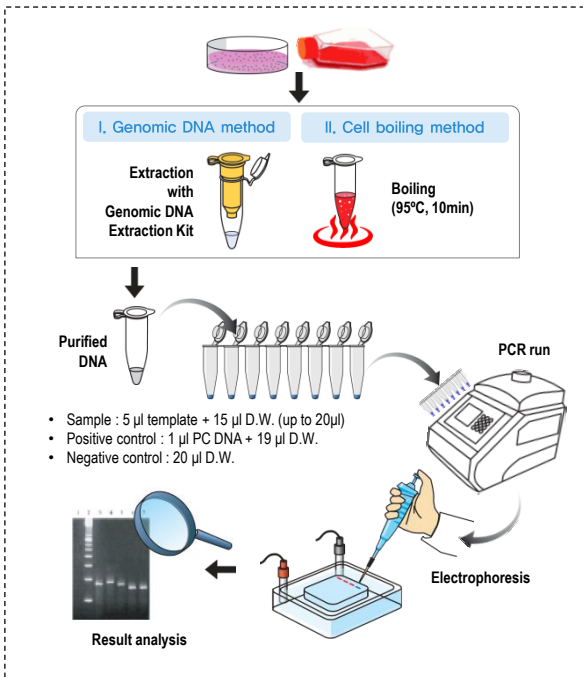
- e-Myco™ plus Mycoplasma PCR Premix : Blue colored pellet in PCR Strip
- Control DNA : Colorless and transparent liquid
- DNase/RNase Free Water : Colorless and transparent liquid

KIT CONTENTS, PACKAGING / STORAGE INFORMATIONS

No	Contents	Composition	25237	25238
1	e-Myco™ plus Mycoplasma PCR Premix	< 0.01% Hot start Taq DNA Polymerase < 0.01% dATP, dTTP, dGTP, dCTP < 0.005% Mycoplasma Primers, Internal Control < 0.001% 8-MOP (dissolved in DMSO)	48T	8T
2	Control DNA	< 0.01% recombinant DNA included partial 16S sequence of <i>M. hyorhinis</i>	25 µl x 3T	25 µl x 1T
3	DNase/RNase Free Water	No template control < DNase/RNase Free Water	1 ml x 1T	0.2 ml x 1T

- Storage condition** : Store the product at -22 ~ -18°C after receiving.
- Expiration** : e-Myco™ plus Mycoplasma PCR Detection Kit can be stored for up to 12 months without showing any reduction in performance and quality under appropriate storage condition. The expiration date is labeled on the product box.

OVERVIEW OF MYCOPLASMA DETECTION



- Sample : 5 µl template + 15 µl D.W. (up to 20µl)
- Positive control : 1 µl PC DNA + 19 µl D.W.
- Negative control : 20 µl D.W.

SAMPLE PREPARATION

※ Protocol I : Genomic DNA Extraction Method

- PCR inhibiting substances may accumulate over time in cell culture medium.
- Medium with more than 10-12 % serum has inhibitory effects on downstream application such as PCR. Moreover, phenol red, a routine material in cell culture medium, is likely to cross-react and thus interfering the signals in PCR.
- These negative effects can be overcome by using the G-spin™ Total DNA Extraction Mini Kit for Sample preparation.
- For this reason, it is recommended to isolate genomic DNA from samples purely to ensure accuracy and repeatability of analysis.

- Prepare 200 µl of cell culture material then transfer into a new 1.5 ml microtube
- Add 200 µl Buffer ML1, 20 µl Proteinase K and 5 µl RNase A Solution into sample tube and mix thoroughly by inverting or pipetting.
- Incubate the lysate at 56°C (preheated heat block or water bath) for 10 min.
- After lysis completely, add 200 µl of Buffer ML2 into upper sample tube and mix thoroughly.
- Add 200 µl of absolute ethanol into the lysate, and mix well by gently inverting 5 - 6 times or by pipetting. DO NOT vortex. After mixing, briefly centrifuge the 1.5 ml tube to remove drops from inside of the lid.
- Carefully transfer the entire lysate to the Spin Column (in a 2 ml Collection Tube) without wetting the rim, close the cap, and centrifuge at 13,000 rpm for 1 min. Discard the filtrate and place the Spin Column in a 2 ml Collection Tube (reuse).
- Add 700 µl of Buffer MWA to column and centrifuge for 1 min at 13,000rpm.
- Add 700 µl of Buffer MWB to the Column without wetting the rim, and centrifuge for 1 min at 13,000 rpm. Discard the flow-through and place the Column into a 2.0 ml Collection Tube (reuse). Then again centrifuge for additionally 1 min to dry the membrane. Discard the flow-through and Collection Tube altogether.
- Place the Spin Column into a new 1.5 ml tube (not supplied), and 50 µl of Buffer ME directly onto the membrane. Incubate for 1 min at room temperature and then centrifuge for 1 min at 13,000 rpm to elute.

※ Protocol II : Boiling Method

- Prepare cell suspensions from the test cell culture in a 1.5 ml tube. Then count cell numbers by general counting methods. You need at least 5x10⁴ cells per test.
Note : Strong mycoplasma infections are detected in as little as 10-100 cells, while weak infections require cells over 5,000-50,000 cells. You can dilute the template according to the infection rates you suspect. We recommend that you perform the PCR reaction after preparing serial dilutions of the straight supernatant to obtain optimal results.
- Transfer the counted cells (over 5x10⁴ cells) to a 1.5 ml tube. Spin the tube in a microcentrifuge for 10-15 seconds. Carefully decant the supernatant.
- Resuspend the cells in 1 ml of sterile PBS or DPBS solution for washing.
- Spin the tube in a microcentrifuge for 10-15 seconds. Carefully decant the supernatant. [Option] Repeat this wash step once more.
- Resuspend the cell pellets in 100 µl of sterile PBS or DPBS solution.
Note : If you want the best result, use of PBS solution is better than Tris (10 mM, pH 8.5), TE (10 mM Tris, 0.1 mM EDTA), or autoclaved DW.
- Heat the samples at 95 °C for 10 min, and vortex for 5-10 sec. Then, centrifuge for 2 min at 13,000 rpm with a tabletop centrifuge (at RT).
- Transfer an aliquot of the heated supernatant to a fresh tube. This supernatant will be used as the template in the PCR.

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English (문/英讀)

Instruction For Use

Beief & Beief, LiliF Diagnostics MDX Kit

