iNtRON Biotechnology
(# 701~704, Jung-Ang Industria V, Sangdaewon-dong)
137, Sagimakgol-ro, Jungwon-gu,
Sungnam-si, Gyeonggi-do, Korea

e-Myco™ Mycoplasma PCR Detection Kit (ver. 2.0)

RUO Research Use only

REF 25235 Σ48 REF 25236 Σ8 -22 ℃ -18 ℃

Test for the detection of Mycoplasmas by PCR analysis

BACKGROUND INFORMATION

Mycoplasma are common and serious contaminants of cell cultures. It has been shown that 30% to 87% of cell cultures are infected with mycoplasma. Many mycoplasma contaminations, particularly in continuous cell lines, grow slowly and do not destroy host cells but are still able to affect various parameters, leading to unreliable or false results. These effects include changes in metabolism, growth, viability, DNA, RNA, and protein synthesis, and morphology. Testing for mycoplasma is an essential quality control tool to assure accurate research and reliable biotechnological products.

The e-Myco™ (ver.2.0) product is a set of primers designed to detect the presence of mycoplasma that might contaminate biological materials such as cultured cells. Conventional techniques that are used to detect mycoplasma involve culturing samples on selective media, which needs at least 1 week to obtain results, whereas by performing PCR with this primer set, which is based on conserved 16S rRNA, detection results are obtained in a few hours. Furthermore, if you want to know the detailed species, you may perform PCR and sequencing from your designed primers. The adopted 8-methoxypsoralen (8-MOP) is used to extinguish the template activity of contaminated DNAs. 8-MOP is known to intercalate into double-stranded nucleic acids and form a covalent interstrand crosslink after photo-activation by incident light at wavelength 320-400 nm. An 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 sample primer pair was used to amplify the internal control and target DNA, which were differentiated by size. Each tube of the e-Myco™ Mycoplasma PCR Detection Kit (ver.2.0) contains all the components for PCR except for template: i-StarTaqTM DNA Polymerase, dNTPs, 10x Buffer, primers, 8-MOP, and internal control for mycoplasma partial gene amplifications. So, you can just add your templates and perform the PCR reaction.

CHARACTERISTICS

- **Premix Type :** This e-Myco™ Mycoplasma PCR Detection Kit (ver.2.0) contains all the components for the PCR reaction. You just add template DNA or samples.
- **Broad Species Detection :** You can detect five common cell culture-infecting species of mycoplasma and also other various species of mycoplasma
- **Species Determination :** You can determine the species of mycoplasma by sequencing the amplified PCR products.
- **Internal Control :** The exogenous internal positive control embedded in the product prevents misjudgment that possibly arises from an erroneous PCR test.
- **Elimination of Carryover Contamination System :** 8-MOP solution prevents carryover contamination by PCR products.

INTENDED USE

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

e-Myco™ Mycoplasma PCR Detection Kit (ver 2.0) 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)
- Thermal cycler
- Disposable gloves
- Myco-Spin Mycoplasma Extraction Kit
- Vortex mixer
- Heat block

DESCRIPTION

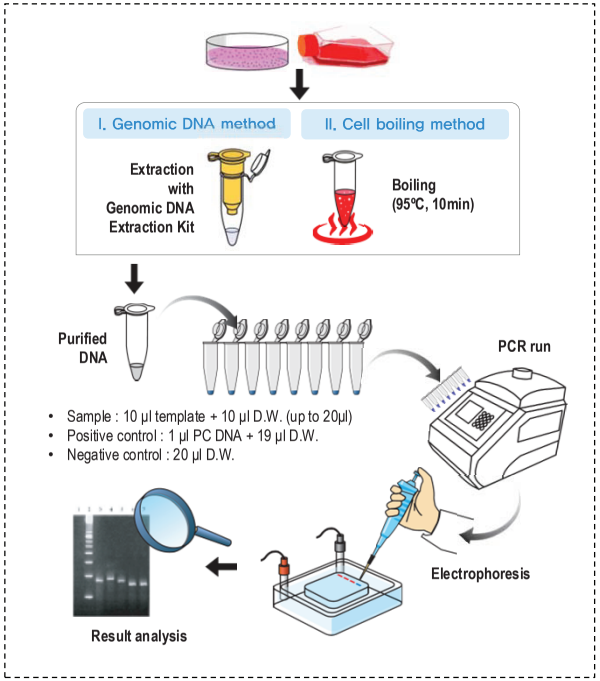
- e-Myco™ Mycoplasma PCR Premix : Blue colored pellet in PCR Strip
- Control DNA : Colorless and transparent liquid
- DNase/RNase Free Water : Colorless and transparent liquid

KIT CONTENTS, PACKAGING / STORAGE INFORMATIONS

No	Contents	Composition	25235	25236
1	e-Myco™ 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% genomic DNA extracted from cultured human cell contaminated with *M. hyorhinis*.	25 µl x 3T	25µl 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℃ after receiving.
- Expiration : e-Myco™ Mycoplasma PCR Detection Kit (ver 2.0) 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 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 overcame by using the Myco-Spin Mycoplasma Extraction Kit for Sample preparation.
- For this reason, it is recommended to isolate genomic DNA from samples purely to ensure accuracy and repeatability of analysis.

1. **Prepare 200 µl of cell culture material then transfer into a new 1.5 ml microtube**
2. **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.**
3. **Incubate the lysate at 56℃ (preheated heat block or water bath) for 10 min.**
4. **After lysis completely, add 200 µl of Buffer ML2 into upper sample tube and mix thoroughly.**
5. **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.**
6. **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).**
7. **Add 700 µl of Buffer MWA to column and centrifuge for 1min at 13,000rpm.**
8. **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.**
9. **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

1. **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 $5x10^4$ 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.
2. **Transfer the counted cells (over $5x10^4$ cells) to a 1.5 ml tube. Spin the tube in a microcentrifuge for 10~15 seconds. Carefully decant the supernatant.**
3. **Resuspend the cells in 1 ml of sterile PBS or DPBS solution for washing.**
4. **Spin the tube in a microcentrifuge for 10~15 seconds. Carefully decant the supernatant.** [Option] Repeat this wash step once more.
5. **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.
6. **Heat the samples at 95 ℃ for 10 min, and vortex for 5-10 sec. Then, centrifuge for 2 min at 13,000 rpm with a tabletop centrifuge (at RT).**
7. **Transfer an aliquot of the heated supernatant to a fresh tube. This supernatant will be used as the template in the PCR.**

Belief & Relief, LiliF Diagnostics MDx Kit
Instruction For Use
English (영문 英語)

LiliF Diagnostics

PCR TEST PROTOCOLS

※ Precautions before Testing

- Leave it at 4℃ or room temperature for thawing. Do not leave it at room temperature more than 1 hour.
- Use clean, disposable gloves when performing the assay and make sure that the work area is clean prior to starting the assay setup.
- All procedures must be done on a clean bench that should be cleaned with 70% alcohol or 10% household bleach (Na-hypochlorite) after use. The samples used should be kept separate. If discarded, it is considered to be a biological hazardous substance after high-pressure sterilization and discarded.

※ Test Procedure

1. **Prepare appropriate number of e-Myco™ Mycoplasma PCR Premix tubes.**
   ⚠ An appropriate number of tubes means the combination of two tubes in the number of samples, which includes a positive control and a negative control.
2. **Add 10 µl of DNase/RNase-free water into the RT-PCR Pre-mixture tube.**
3. **Add 10 µl of DNA sample to each of strip tubes.**
4. **For positive and negative confirmation, use 1 µl of positive control or DNase/RNase Free water as a test sample. Then, adjust the reaction volume to 20 µl.**
5. **Dissolve the blue pellet by pipetting or vortexing.**
   ⚠ The pellet is easily dissolved, by letting the mixture stand at R.T. for 1-2 minutes after adding water.
6. **Perform PCR reaction of samples as the below process using thermal cycler.**

PCR Condition		Temp	Time
Initial denaturation		94 ℃	1 min
X 35 Cycle	Denaturation	94 ℃	30 sec
	Annealing	60 ℃	20 sec
	Extension	72 ℃	1 min
Final extension		72 ℃	5 min

7. **For analysis by electrophoresis, use 5 µl of each tube.**
8. **PCR products should be discarded after UV irradiation (10 min) to prevent carry-over contamination.**
   ⚠ Contamination of DNA is a serious problem of PCR. Please discard PCR products after UV irradiation (365 nm) to prevent carry-over contamination.

TECHNICAL INFORMATION

※ PCR product size

The size of DNA fragments that are amplified by the specific primers in this kit is **about 270 bp**. However, the sizes of PCR product differ slightly from species to species (268 bp~277 bp). You can confirm by sequencing analysis after T/A vector cloning and other cloning methods.

※ Additional Informations

- This e-Myco™ Mycoplasma PCR Detection Kit (ver.2.0) will provide a sensitive performance to detect mycoplasma contamination in cell lines. Under optimal conditions, templates derived from supernatants of an infected cell culture will yield a maximum signal in the PCR reaction, whereas an uninfected cell line will yield no PCR products. Undoubtedly, there will be variations in cell numbers, infection amount, and templates that may contribute to signal differences in your experiments.
- It is recommended that you use cultured cells that have cultivated for 3~6 days after subculturing as a sample for mycoplasma detection. You may not detect mycoplasma infection efficiently when you use cells that are not or shortly cultivated.
- The PCR amplification efficiency varies by mycoplasma infection range. Strong mycoplasma infections are detected in as little as 10~100 cell equivalents, while weak infections require cell equivalents from the 5000~50,000 range. So, we recommend that you plan various cell numbers in preparing PCR templates from the cultured cells by using the boiling method. Please refer to Fig. 2.
- If you perform genetic analysis for determining more detailed species, please extract the DNA and apply it to the PCR process. We recommend that you use our Myco-Spin Mycoplasma Extraction Kit (Cat. No. 17541).

※ Mycoplasma Detection limit

- K562 cell (M. fermentans-infected) : small cell numbers, such as 12 cells
- K562 gDNA (M. fermentans-infected) : small quantities, such as 3.25 pg
- M. fermentans : small copy numbers, such as 20 cfu/ml

1) Result for the various concentration of template DNA

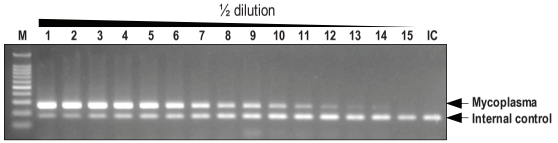


Fig.1. Mycoplasma detection was performed for genomic DNA

Genomic DNA was isolated from *M. fermentans*-infected K562 using genomic DNA extraction kit. The isolated gDNA was serially diluted for PCR of mycoplasma detection. **These results show that it can be applied to mycoplasma detection with small quantities, such as 6.3 ~ 3.25 pg of gDNA**

Lane M, 100bp DNA Marker; **lane IC**, Internal control ,**lane 1**, 50 ng; **lane 2**, 25 ng; **lane 3**, 12.5 ng; **lane 4**, 6.3 ng; **lane 5**, 3.2 ng; **lane 6**, 1.6 ng; **lane 7**, 800 pg; **lane 8**, 400 pg; **lane 9**, 200 pg; **lane 10**, 100 pg; **lane 11**, 50 pg; **lane 12**, 25 pg; **lane 13**, 12.5 pg; **lane 14**, 6.3 pg; **lane 15**, 3.25 pg

2) Result for the various cell number

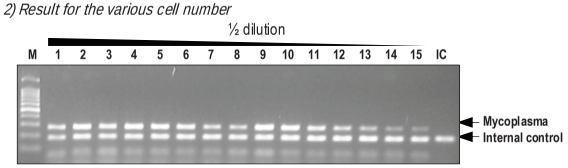


Fig.2. Mycoplasma detection was performed using the *e*-Myco™ Mycoplasma PCR Detection Kit (ver.2.0) method

Mycoplasma detection from cell lysates of *M. fermentans*-infected K562 using the *e*-Myco™ Mycoplasma Detection Kit (ver.2.0). The *M. fermentans*-infected K562 cells were serially diluted for PCR of mycoplasma detection and then PCR was performed per the e-Myco™ Kit 's protocol. **These results show that it can be applied to the mycoplasma detection with small cell numbers, such as 12 cells**

Lane M, 100bp DNA Marker; **lane IC**, Internal control; **lane1**, $2x10^5$; **lane 2**, $1x10^5$; **lane 3**, $5x10^4$; **lane 4**, $2.5x10^4$; **lane 5**, $1.25x10^4$; **lane 6**, $6.25x10^3$; **lane 7**, $3.125x10^3$; **lane 8**, $1.56x10^3$; **lane 9**, $7.8x10^2$; **lane 10**, $3.9x10^2$; **lane 11**, $1.9x10^2$; **lane 12**, 96; **lane 13**, 48; **lane 14**, 24; **lane 15**, 12

※ Elimination of Carryover Contamination

1) 1st PCR

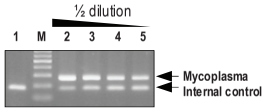


Fig. 3. Mycoplasma detection was performed for genomic DNA

Lane M, 100bp DNA Marker; **lane 1**, Internal control; **lane 2**, 25pg; **lane 3**, 12.5pg; **lane 4**, 6.3pg; **lane 5**, 3.25pg

2) 2nd PCR

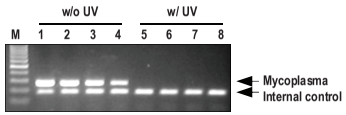


Fig. 4. 8-MOP activation by UV irradiation (10min) of 1st PCR template

Panel w/o UV, without UV irradiation; **panel w/UV, with UV irradiation (10 min); lane M,** 100bp DNA Marker ; **lane 1**, PCR product (1 µl) used from fig. 3. lane 2; **lane 2**, PCR product (1 µl) used from fig. 3. lane 3; **lane 3** , PCR product (1 µl) used from fig.3. lane 4; **lane 4**, PCR product (1 µl) used from fig. 3. lane 5; **lane 5**, PCR product (1 µl) used from fig. 3. lane 2; **lane 6**, PCR product (1 µl) used from fig. 3. lane 3; **lane 7**, PCR product (1 µl) used from fig. 3. lane 4; **lane 8**, PCR product (1 µl) used from fig. 3. Lane 5

※ SPECIES DETERMINATION BY SEQUENCING ANALYSIS

- The sequence of amplified PCR products differs slightly from species to species. You can determine approximately the Mycoplasma species by sequencing analysis with the following primers. Please refer to the phylogenetic table on the next page. For more detailed species analysis, you should perform additional PCR reactions with your designed primers.
- We list only the Forward primer sequences. Please synthesize the primer, and then analyze by general sequencing methods.
- Sequencing primer sequences : AGGAT TAG ATA CCC TGG TAG TC-3' (20 mer)
  ⚠ The PCR primers used in this kit differ from the sequencing primers. We do not list the PCR primer sequences contained in this kit.

※ TROUBLESHOOTING GUIDE

Symptoms	Possible Causes	Comments & Suggestions
No Target band in positive reaction	Check internal control band	• If internal control band is seen, PCR has been performed properly; it is not a problem of the product.
	Check the quality or concentration of template	• If the PCR reaction is inhibited by impurities included in DNA preparation, the use of diluted DNA as a template may be helpful. • Whereas the signals of internal control (app. 160 bp length) are shown, if the target band is not shown, it indicates that the sample is not infected by Mycoplasma
	Check a PCR machine	• The problem can be caused by the PCR machine. Please check the temperature and make sure to check that the machine is working properly.
No internal control band	Check template concentration	• Competition can occur by using high concentrated DNA template. Please repeat the PCR with a diluted template. If the concentration of template is above 50 ng, the signal of internal control may be disappeared by competition with the template.
	Check the quality of template (possibility of contamination with PCR inhibitors)	• If the PCR reaction is inhibited by impurities included in DNA preparation, the use of diluted DNA as a template may be helpful. If there is no internal control band, please inquire with our technical support staff.
	Check the storage condition of product.	• Keep appropriate preservation conditions
Presence of amplified product in the negative control	Check contamination of D.W.	• D.W. can be contaminated. Perform PCR again with fresh sterile water
	Check contamination of lab instruments and other environments	• We recommend that you use filter tips to reduce contamination and that you use a pipette after sterilization. All procedures should be done in sterilized conditions.
Poor resolution on agarose gel	Low gel concentration	• We recommend to use a 1.5~2% agarose gel. • Check the resolution comparing with DNA marker
	Short running time	• We recommend that electrophoresis is performed for 40 min at 100 V/14 cm using a 6 cm long 2% agarose gel.

ORDERING INFORMATION

Product Name	Amount	Cat. No.
Myco-Spin Mycoplasma Extraction Kit	50 Col.	17541
M-Solution™ 1-2 Antibiotic for Mycoplasma	10 ml (each)	21081
SiZer™-100 DNA Marker	0.5 ml	24073

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EXPLANATION OF SYMBOLS

LOT	Batch number	IVD	In vitro diagnostic	REF	Product number
Σ	Sufficient for tests	⊘	Do not reuse		Storage temperature limitation
	Manufacturing date		Expire date		Keep away from sunlight
	Manufactured by	⚠	Attention	i	Consult Instructions For Use

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