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

Test for the detection of Mycoplasmas by PCR analysis

e-Myco™ plus Mycoplasma PCR Detection Kit

RUO Research Use Only | REF 25237 | Σ 48 | -20℃

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 is designed to detection mycoplasma contamination that appears during the cell culture process.

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.

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-methoxypsoralen (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 D.W.
- **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)
- Thermal cycler
- Disposable gloves
- G-spin™ Total DNA Extraction Kit
- Vortex mixer
- Heat block

MATERIAL

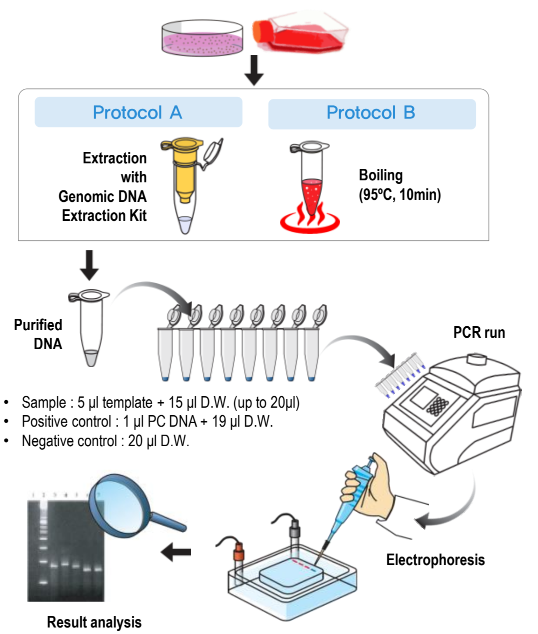
- e-Myco™ plus Mycoplasma PCR Premix : Blue colored pellet in PCR Strip
- Positive Control : Colorless and transparent liquid
- DNase/RNase Free Water : Colorless and transparent liquid

KIT CONTENTS, PACKAGING / STORAGE INFORMATIONS

No	Contents	Composition	25237
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
2	Positive Control	< 0.01% recombinant DNA included partial 16S sequence of *M. hyorhinis*	25 µl x 3Tubes
3	DNase/RNase Free Water	No template control < DNase/RNase Free Water	1 ml x 1Tube

- Storage condition : Store the product at below -20℃ after receiving.
- Expiration : e-Myco™ plus Mycoplasma PCR Detection Kit can be stored for up to 18 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

You can use this protocol just for detecting the contamination of mycoplasma. However, if you want to perform genotyping for the detailed determination of species, please purify the genomic DNA of suspected Mycoplasma-infected cells using our G-spin™ Total DNA Extraction Kit (Cat.No.17045). You may use simply this protocol or your other general boiling methods.

[TECHNICAL TIP]
1. Use clean, disposable gloves when performing the assay and make sure that the work area is clean prior to starting the assay setup.
2. Keep your reagents and PCR mixture tubes on a cold block during reaction setup.
3. Use positive displacement pipettes.
4. The amplification and preparation areas should be physically separated.

※ Preparing samples for DNA extraction

1. **Prepare cell suspensions from cell culture in a 1.5 ml clean tube. Then, count cell numbers by general counting methods. You need at least 5x10^4 cells per test.**

   ⚠ Harvest adherent cells with trypsin-EDTA buffer using standard techniques. Pipette 1 ml of trypsin-EDTA treated adherent cells. Generally, with suspension cells you need not treat with trypsin-EDTA buffer. We recommend that you count the cells. You should prepare at least 5x10^4 cells per test.

   ⚠ 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

2. **Transfer the counted cells (over 5x10^4 cells) to a 1.5 ml clean tube. Spin the tube in a microcentrifuge for 10~15 seconds. Carefully discard the supernatant.**

※ PROTOCOL A : Using genomic DNA extraction method

We recommend using the G-spin™ Total DNA Extraction Kit (iNtRON, Cat No. 17045), which can extract genomic DNA from the pelleting cell. When using this product, extract genomic DNA from the pelleting cell by complying with protocol C (iNtRON, Cat No. 17045) or you can use the extraction kit you are using.

⚠ Mycoplasma is a genus of bacteria that grows by being parasitic on a host cell and survives independently only under mycoplasma-specific growth conditions. Therefore, in order to test mycoplasma in a cell culture medium, you can extract genomic DNA from the pelleting cell (refer to paragraphs 1 and 2 of "Preparing samples for DNA extraction").

※ PROTOCOL B : Using the Boiling method

1. **For extracting of genomic DNA from samples, refer to paragraphs 1 and 2 of "Preparing samples for DNA extraction".**
2. **Resuspend the cells in 1 ml of sterile PBS or DPBS buffer for washing.**
3. **Spin the tube in a microcentrifuge for 10~15 seconds. Carefully discard the supernatant.**

   ⚠ **[Option]** Repeat this wash step once more to reduce the unwanted PCR inhibition.

4. **Resuspend the pellet in 100 µl of sterile PBS or DPBS buffer.**

   ⚠ For the negative control, use 5 µl DNase / RNase Free Water instead of the genomic sample and 5 µl of Positive Control DNA sample included in the kit for positive control

   ⚠ If you want the best result, use of PBS buffer is better than Tris buffer(10 mM, pH 8.5), TE buffer (10 mM Tris, 0.1 mM EDTA), or autoclaved D.W.

5. **Incubate the sample at 95 ℃ for 10 mins, and gently vortex for 5-10 sec. Then, centrifuge the sample for 2 min at 13,000 rpm with a tabletop centrifuge (at RT).**
6. **Transfer an aliquot of the boiled supernatant to a fresh tube. This supernatant will be used as the template in the PCR. (Store the sample for up to 7 days at 2+~8C or at -18~20C for long term storage.)**

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™ plus 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 15 µl of DNase/RNase-free water into the PCR pre-mixture tube.**
3. **Add 5µl 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	58 ℃	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 mins) 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

※ Interpretation

- **Sample control :** a parameter indicating the appropriateness in sample preparation
- **Target band :** a parameter of mycoplasma infection
- **Internal control :** a parameter checking any problems that may arise during amplification

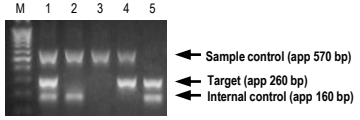


Fig. 1. Recommended sample amount of e-Myco™ plus Mycoplasma PCR Detection Kit

Lane	Mycoplasma	Test case description	Template amount
1	Contamination	Optimal	1 ~ 50 ng
2	Free	Optimal	1 ~ 50 ng
3	Free	Excess template	> 50 ng
4	Contamination	Excess template	> 50 ng
5	Contamination	Small amount of template	1 ng

※ Minimal amount of genomic DNA detectable

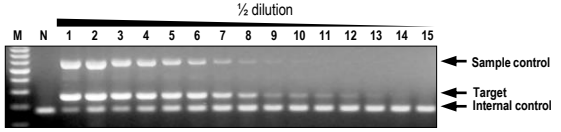


Fig. 2. Result of determining minimal required amount of genomic DNA per test
To determine the minimal required amount of genomic DNA, genomic DNA was isolated from a pure culture of M. fermentans-infected K562 cells using genomic DNA extraction kit. The isolated genomic DNA was serially diluted for PCR detection. **The result indicates that the detection limit with this kit is 10 ~ 20 pg of genomic DNA per test.**

Lane	M	N	1	2	3	4	5	6
gDNA	100 bp DNA Marker	0 ng	100 ng	50 ng	25 ng	12.5 ng	6.3 ng	3.2 ng
Lane	7	8	9	10	11	12	13	14	15
gDNA	1.6 ng	800 pg	400 pg	200 pg	100 pg	50 pg	25 pg	12.5 pg	6.25 pg

※ Minimal cell number required

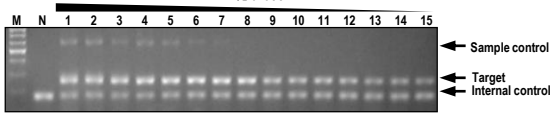


Fig. 3. Result of determining minimal required cell number per test
To determine the minimal required cell number, M. fermentans-infected K562 cells were grown in pure culture, serially diluted and tested. **The result indicates that the detection limit with this kit is 15 cells per test**.

Lane	M	N	1	2	3	4	5	6
gDNA	100 bp DNA Marker	0	$2.5x10^5$	$1.25x10^5$	$6.25x10^4$	$3.12x10^4$	$1.56x10^4$	$7.8x10^3$
Lane	7	8	9	10	11	12	13	14	15
gDNA	$3.9x10^3$	$1.9x10^3$	$9x10^2$	$4.8x10^2$	$2.4x10^2$	120	60	30	15

※ DETECTABLE MYCOPLASMA STRAINS (8 Genus / 209 Species)

Genus	Species
Acholeplasma (5)	*Acholeplasma granularum* *Acholeplasma laidlawii* *Acholeplasma modicum* *Acholeplasma morum* *Acholeplasma oculi*
Anaeroplasma (3)	*Anaeroplasma abactoclasticum* *Anaeroplasma bactoclasticum* *Anaeroplasma varium*
Asteroleplasma (1)	*Asteroleplasma anaerobium*
Entomoplasma (5)	*Entomoplasma lucivorax* *Entomoplasma luminosum* *Entomoplasma melaleucae* *Entomoplasma somnilux* *Entoplasma ellychniae*
Mycoplasma (182)	*M. adleri* *M. agalactiae* *M. agalactiae (strain PG2)* *M. agassizii* *M. alkalescens* *M. alligatoris* *M. alvi* *M. amphoriforme* *M. anatis* *M. anseris* *M. arginini* *M. arthritidis* *M. auris* *M. bovigenitalium* *M. bovirhinis* *M. bovis* *M. bovoculi* *M. buccale* *M. buteonis* *M. californicum* *M. canadense* *M. canimucosale* *M. canis* *M. capricolum* *M. capricolum subsp. capricolum* *M. hyophayngis* *M. hyopneumoniae* *M. caviae* *M. caviphayngis* *M. citelli* *M. cloacale* *M. coccoides* *M. collis* *M. columbinasale* *M. columbinum* *M. columborale* *M. conjunctivae* *M. corogypsi* *M. cottewii* *M. cricetuli* *M. crocodyli* *M. cynos* *M. dispar* *M. edwardii* *M. elephantis* *M. equigenitalium* *M. equirhinis* *M. erythrodidelphis* *M. falconis* *M. fastidiosum* *M. faucium* *M. felifaucium* *M. feliminutum* *M. felis* *M. fermentans* *M. flocculare* *M. gallinaceum* *M. gallinarum* *M. gallisepticum* *M. gallopavonis* *M. gateae* *M. genitalium* *M. genitalium G37* *M. glycophilum* *M. gypis* *M. haemocanis* *M. haemofelis* *M. haemolama* *M. haemomuris* *M. hominis* *Mycoplasma sp. Ms01* *Mycoplasma sp. Ms02* *Mycoplasma sp. Ms03* *M. hyopneumoniae (strain 232)* *Mycoplasma sp. PG50* *M. hyopneumoniae (strain7448)* *M. insons* *M. hyorhinis* *M. lagogenitalium* *M. imitans* *M. hyosynoviae* *M. indiense* *M. iguanae* *M. iowae* *M. iners* *M. leocaptivus* *M. leonicaptivi* *M. leopharyngis* *M. lipofaciens* *M. lipophilum* *M. microti* *M. moatsii* *M. mobile* *M. molare* *M. monodon* *M. muris* *M. mustelae* *M. Mycoides* *M. mycoides subsp. capri* *M. mycoides subsp. mycoides LC* *M. mycoides subsp. mycoides SC* *M. mycoides sunsp. capri* *M. neurolyticum* *M. opalescens* *M. orale* *M. ovipneumoniae* *M. oxoniensis* *M. penetrans* *M. phocicerebrale* *M. phocidae* *M. phocirhinis* *M. pirum* *M. pneumoniae* *M. primatum* *M. pullorum* *M. pulmonis* *M. putrefaciens* *M. salivarium* *M. simbae* *M. spermatophilum* *M. maculosum* *M. meleagridis* *M. sphenisci* *M. spumans* *M. sturni* *M. suali* *M. subdolum* *M. suis* *M. synoviae* *M. synoviae (strain 53)* *M. testudineum* *M. testudinis* *M. timone* *M. verecundum* *M. vulturii* *M. wenyonii* *M. yeatsii* *M. zalophi* *M. zalophidermidis* *Mycoplasma sp. Saa1e* *Mycoplasma sp. Z61* *Mycoplasma sp. SF12* *Mycoplasma sp. 07SH-h* *Mycoplasma sp. 07SH-p* *Mycoplasma sp. 10T3* *Mycoplasma sp. 10T4* *Mycoplasma sp. 11CL2* *Mycoplasma sp. 1220* *Mycoplasma sp. 13CL* *Mycoplasma sp. 15CL2* *Mycoplasma sp. 23fIAT* *Mycoplasma sp. 2F1AT* *Mycoplasma sp. 34CL* *Mycoplasma sp. 39CL* *Mycoplasma sp. 50587* *Mycoplasma sp. 8790CV* *Mycoplasma sp. 94630* *Mycoplasma sp. A1802T* *Mycoplasma sp. ARNO* *Mycoplasma sp. 'bovine group 7'* *Mycoplasma sp. C3T* *Mycoplasma sp. China-1* *Mycoplasma sp. CSL 4779* *Mycoplasma sp. CSL 7518-lung* *Mycoplasma sp. VJC358* *Mycoplasma sp. HRC689* *Mycoplasma sp. IS2505* *Mycoplasma sp. M1* *Mycoplasma sp. M200-2* *Mycoplasma sp. M209-7* *Mycoplasma sp. M209-8* *Mycoplasma sp. M221-9* *Mycoplasma sp. M222-2* *Mycoplasma sp. M222-5* *Mycoplasma sp. M26* *M. capricolum subsp. capripneumoni* *M. capricolum subsp. capripneumoniae* *Mycoplasma . sp. ovine/caprine serogroup 11* *M. hyopneumoniae (strain J / ATCC 25934)* *Mycoplasma. sp. 'feline hemotropic Switzerland'* *Mycoplasma sp. Saa1c* *Mycoplasma sp. SF9*
Mesoplasma (3)	*Mesoplasma entomophilum* *Mesoplasma florum* *Mesoplasma lactucae*
Spiroplasma (9)	*Spiroplasma apis* *Spiroplasma citri* *Spiroplasma CN-5* *Spiroplasma DU-1* *Spiroplasma DW-1* *Spiroplasma gladiatoris* *Spiroplasma mirum* *Spiroplasma MQ-1* *Spiroplasma taiwanense*
Ureaplasma (1)	*Ureaplasma urealyticum*

※ IMPORTANT NOTES

- 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 : GGA TTA GAT ACC CTG GTA GTC CAC G-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.
- The PCR conditions were optimized to obtain the highest level of sensitivity of target gene detection. So, the internal control band or sample control band may be sometimes disappeared depending on the efficiency of target gene amplification. The efficiency of the target gene amplification is dependent upon the amount of template DNA added to the reaction. Please refer the following table to show the dependency.

Lane	Amount of template DNA
Optimal conditions (Three bands are appeared)	1 ~ 50 ng of template DNA
Masking point of internal control band	above 50 ng of template DNA
Ending point of sample control band	below 1 ng of template DNA
Limit of sensitivity in target gene amplification	6.3 pg of template DNA

※ 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 sample control (app. 570 bp length) and 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. It does not cause any problem, because the signal of sample control (app. 570 bp length) can function as a internal control.
	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.
No sample control band	Check template concentration	• Sometimes, the sample control band may disappeared when the concentration of DNA template is below 1 ng. Check the quantity of DNA template, and adjust the amount of DNA template in 20 µl PCR reaction to be above 1 ng.
	Check the source of template	• The primers set included in this kit can amplify a human-specific DNA sequence. If the template source is not human cell, the amplification of sample control does not occur.

ORDERING INFORMATION

Product Name	Amount	Cat. No.
G-spin™ Total DNA Extraction Kit	50 Col.	17045
SiZer™-100 DNA Marker	0.5 ml	24073

LiliF Diagnostics

EXPLANATION OF SYMBOLS

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

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