

e-Myco™ plus Mycoplasma PCR Detection Kit

(for 20 µl rxn)

Cat. No. 25234 96 Tests

DESCRIPTION

- 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.
- Mycoplasma cells are physically small (< 1 µm) and are difficult to detect with a conventional microscope. Mycoplasmas may induce cellular changes, including chromosome aberrations, changes in metabolism and cell growth. Severe Mycoplasma infections may destroy a cell line. Several methods for the detection of Mycoplasmas have been published. The testing required by the regulatory authorities is seeding in culture (agar and liquid media). This test is complicated, time consuming (about 3 ~ 5 weeks), and some Mycoplasma species are difficult to detect with this method. In recent years, the disadvantages of these methods have been acknowledged (such as sensitivity, specificity and long and complex procedures), and use of PCR for the detection of contaminations in cell cultures has become increasingly widespread.
- e-Myco™ plus Mycoplasma PCR Detection Kit greatly simplify 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 Kit 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 was 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 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 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 known to intercalate into double-stranded nucleic acids and form a covalent inter-strand crosslink after photo-activation by incident light at wavelength 320-400 nm, so it is helpful to prevent cross-contamination by PCR products from earlier experiments.
- Each tube of the e-Myco™ plus Mycoplasma PCR Detection Kit contains all the components for PCR except for template: DNA Polymerase, dNTPs, PCR Buffer, primers set, 8-MOP, and internal control. So, you can just add your templates and perform the PCR reaction.

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 (See Technical Guide).
- 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.

APPLICATIONS

- The kit is used for the detection of Mycoplasma species that are most commonly encountered in cell culture, including *M. arginini*, *M. fermentans*, *M. hyorhinis*, *M. orale*, and *Acholeplasma laidlawii*. Furthermore, a most kinds of mycoplasmas can be detected easily (See Technical Guide).

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

KIT CONTENTS and STORAGE

Label	Contain
e-Myco™ plus Mycoplasma PCR Detection Kit	96 Tubes
Control DNA (genomic DNA from <i>M. fermentans</i> -infected K562)	30 µl (20 ng/µl)
DNase/RNase-free Distilled Water	2 ml

- Store at -20°C. e-Myco™ plus Mycoplasma PCR Detection Kit is a novel vacuum-dried premix type. This product has a stable shelf-life of a minimum of 1 year when stored at -20°C and unopened container.

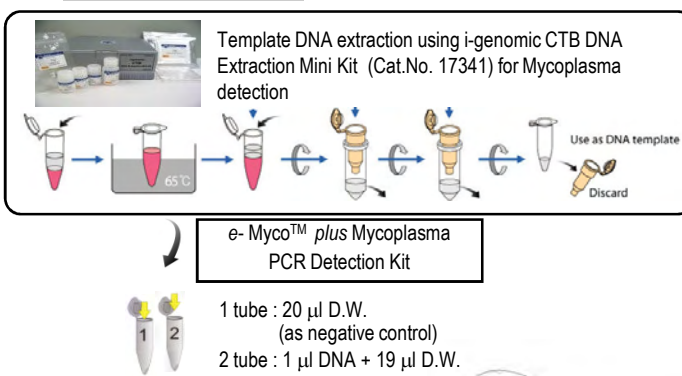
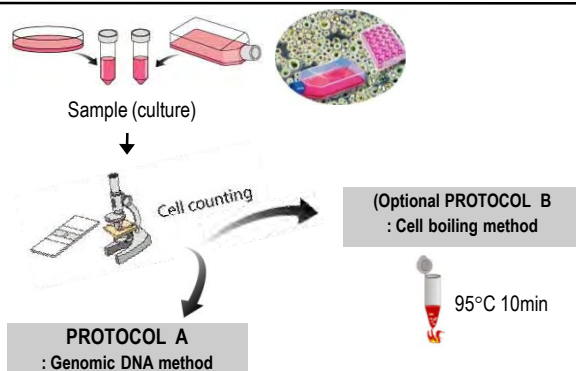
REACTION TUBE COMPONENT

- PCR Reaction volume 20 µl reaction

e-Myco™ plus Mycoplasma PCR Detection Kit	
DNA Polymerase	2.5U
Chemical Stabilizer	1x
Loading Buffer	1x
dNTPs	250 mM each
Tris-HCl (pH 8.3)	10 mM
KCl	50 mM
MgCl ₂	1.5 mM
Mycoplasma Primers Set	
Internal Control	
8-MOP (dissolved in DMSO)	

Dried under iNTRON's instruction

[Overview of Mycoplasma Detection]



TECHNICAL INFORMATION

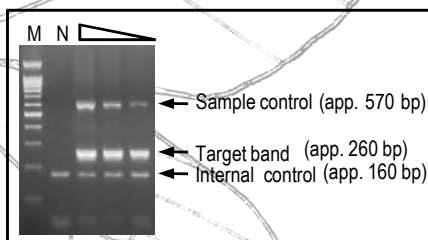
- This e-MycTM plus Mycoplasma PCR Detection Kit offers a sensitive means to detect Mycoplasma contamination in cell lines. Under optimal conditions, templates derived from supernatants of an infected cell culture will yield a maximum signal in the PCR, whereas an uninfected cell line will yield no PCR 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 to use the cultured cells for 3~6 days after sub-culturing as a sample for Mycoplasma detection. You may not detect efficiently Mycoplasma infection when you use the cells that are not or shortly cultivated.
- 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.

	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

* The amount of template DNA is depend on the extent of Mycoplasma infection.

- The efficiency of PCR amplification is varied by the extent of Mycoplasma infection. Strong Mycoplasma infections are detected in a little as 10~100 cell equivalents, while weak infections require cell equivalents in the 5,000~50,000 cell range. So, we recommend to perform PCR reaction with several samples prepared from several cell numbers. Please refer to Fig. 3.
- If you want to do genotyping, excise the target band from the agarose gel, then isolate the DNA fragment using a gel extraction kit(eg. MEGA-spinTM Agarose gel DNA Extraction Kit (Cat. No. 17183), MEGAquick-spinTM PCR & Agarose Gel DNA Extraction Kit (Cat. No. 17282)).

TRIPLE CHECKING SYSTEM



- 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

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 i-genomic CTB DNA Extraction Mini Kit (Cat. No. 17341).

[TECHNICAL TIP]

- Use clean, disposable gloves when performing the assay and make sure that the work area is clean prior to starting the assay setup.
- Keep your reagents and PCR mixture tubes on a cold block during reaction setup.
- Use positive displacement pipettes.
- The amplification and detection areas should be physically separated; i.e., do not use the same bench area to set up the PCR reactions and run your gels.

[CAUTIONS]

- DO NOT expose to UV irradiation, which activates 8-MOP, if you want to determine the detailed species of Mycoplasma by DNA sequencing analysis.

• **PROTOCOL A : Using the genomic DNA extraction kit**

- Prepare the cultured cell according to 1a or 1b.**
 - Cells grown in suspension ; Transfer the culture fluid into 15 ml or 50 ml of centrifuge tube and pellet the culture by centrifugation for 5 min at 3,000 rpm. Remove the supernatant completely and wash the pellet with PBS or fresh media. Then resuspend the washed cell pellet in appropriate volume of PBS or fresh media.
 - Cells grown in monolayer ; Cells grown in monolayer can be detached from culture flask (or plate) by either ¹⁾ Trypsinization or ²⁾ Using a cell scraper.
 - To Trypsinize cells : Remove the medium and wash the cells with preheated (at 37°C) PBS. Then aspirate the PBS and add trypsin solution. After cells have become detached from culture flask (or dish), collect and wash the cells with PBS, then resuspend the washed cell pellet in appropriate volume of PBS or fresh media.
 - Using a cell scrape, detach cells from culture flask or dish. Collect and wash the cells with PBS, then resuspend the washed cell pellet in appropriate volume of PBS or fresh media.
- Determinate the cell number using cell counter (eg. hemocytometer) and transfer the appropriate number of cells (1 ~ 3 x 10⁶ cells) to a new 1.5 ml micro-centrifuge tube.**
- Pellet the cell by centrifugation for 1 min at 13,000 rpm and discard the supernatant. Resuspend the cell pellet with the remaining medium by finger tapping or vortexing.**

Note : In order to ensure efficient lysis, it is essential that the cell pellet are homogeneously resuspended.
- Extract the genomic DNA from the harvested cell. We recommend to use the i-genomic CTB DNA Extraction Mini Kit (Cat. No. 17431) in genomic DNA extraction. The quantity and quality of template DNA used in Mycoplasma detection is important for reliable and acceptable analysis.**
- Add the genomic DNA purified using the i-genomic CTB DNA Extraction Mini Kit (Cat. No. 17431) to each tube of e-MycTM plus Mycoplasma PCR Detection Kit as a template, and then add sterile water to fill the reaction mix up to 20 µl.**

Note: Appropriate amounts of template DNA in a sample: 1 ~ 50 ng
- After sample mixing, perform PCR according to the following procedure.**

Note: We recommend to perform one negative control reaction with sterile water as a template. We also recommend to perform positive control reaction with 0.1 ~ 1 µl of control DNA solution.

	PCR Condition	Temp.	Time
	Initial denaturation	94°C	1 min
35 cycles	Denaturation	94°C	30 sec
	Annealing	58°C	20 sec
	Extension	72°C	1 min
	Final extension	72°C	5 min

- For analysis by electrophoresis, use 5 µl of PCR reaction mixture.**
- PCR products should be discarded after UV irradiation (10 min) to prevent cross-contamination.**

Note: Cross-contamination is a common problem with PCR. Please discard the PCR products after UV irradiation (365 nm) to prevent cross-contamination.

• **(Optional) PROTOCOL B : Using the Boiling Extract 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 1: Harvest adherent cells with trypsin-EDTA solution using standard techniques. Pipette 1 ml of TE-treated adherent cells. Generally, with suspension cells, such as K562, you need not treat with TE solution. We recommend that you count the cells. You should prepare at least 5x10⁴ cells per test (see Technical Guide, >50,000 cells are needed to complete this protocol)

Note 2: Strong Mycoplasma infections are detected in a little as 20 ~ 100 cells, while weak infections require cells over 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 centrifuge 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 centrifuge for 10~15 seconds. Carefully decant the supernatant.**

Note : (Optional) Repeat this washing step once more.

• **(Optional) PROTOCOL B : Using the Boiling Extract Method - continued**

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 for 10 min, and vortex for 5-10 sec. Then, centrifuge for 2 min at 13,000 rpm with a tabletop centrifuge at room temperature.

7. Transfer an aliquot of the heated supernatant to a fresh tube. This supernatant will be used as the template in the PCR.

8. Add 10 µl of the template to each tube of e-Mycos™ plus Mycoplasma PCR Detection Kit, and then add 10 µl of sterile water.

9. After sample mixing, perform PCR reaction according to the procedure presented in protocol A.

Note : We recommend that you perform one negative control reaction with sterile water as a template.

10. For analysis by electrophoresis, use 5 µl of PCR reaction mixture.

11. PCR products should be discarded after UV irradiation (10 min) to prevent cross-contamination.

Note : Cross-contamination is a common problem with PCR. Please discard the PCR products after UV irradiation (365 nm) to prevent cross-contamination.

EXPECTABLE DATA

◆ **Examination of Mycoplasma Infection**

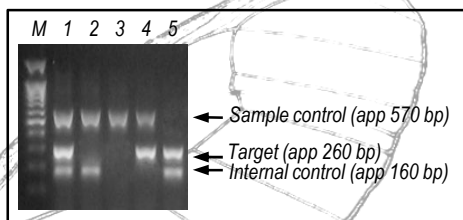


Fig. 1. Exemplary Data

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

TECHNICAL GUIDE

TROUBLESHOOTING GUIDE

1. 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.

2. 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 an 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.

3. 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 mammalian-specific DNA sequence. If the template source is not mammalian cell (eg. Plant, bacteria or Mycoplasma culture), the amplification of sample control does not occur.

4. 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.

5. Poor resolution on agarose gel

- We recommend to use a 1.5~2% agarose gel.
- We recommend that electrophoresis is performed for 40 min at 100 V/14 cm using a 6 cm long 2% agarose gel.

DETECTION LIMITS

Target Gene Detection

- K562 cell (*M. fermentans*-infected) : more than 15 cells
- K562 gDNA (*M. fermentans*-infected) : more than 6.25 pg
- Concentration of Mycoplasma (in the case with *M. fermentans*) : more than 20 cfu/ml

Sample Control Detection

- Human / Mammalian-specific DNA sequence
: app. 570 bp length
: Sample control gene is detectable from above 1 ng of template DNA

Internal Control Detection

- Artificial gene (derived from human TNFα gene)
: app. 160 bp length
: Internal control can serve the tools checking any problems that may arise during amplification. The signal of internal control is mostly appeared. But if the amount of template added is too high (above 50 ng) the signal of internal control may be weakened or disappeared by competition with template DNA.

Experimental Data

1) Minimal amount of genomic DNA detectable

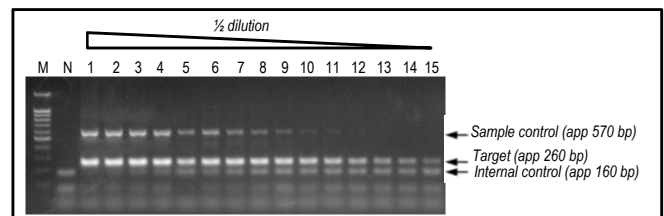


Fig. 2. Result of determining minimal required amount of genomic DNA per test

To determine the minimal required amount of genomic DNA, genomic DNA was isolated from a pure culture of *M. fermentans*-infected K562 cells using i-genomic CTB DNA Extraction Mini Kit (Cat. No. 17431). The isolated genomic DNA was serially diluted for PCR detection. **The result indicates that the detection limit with this kit is 6.3 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

2) Minimal cell number required

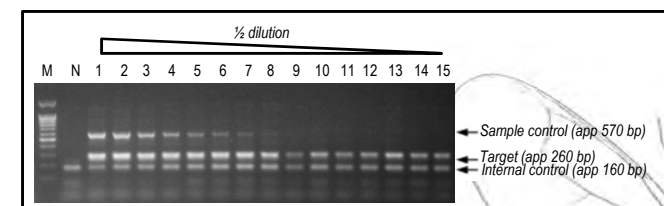


Fig. 3. Result of determining minimal required cell number per test

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	
Cell number	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
Cell number	3.9x10 ³	1.9x10 ³	9x10 ²	4.8x10 ²	2.4x10 ²	120	60	30	15

3) Minimal Mycoplasma concentration detected

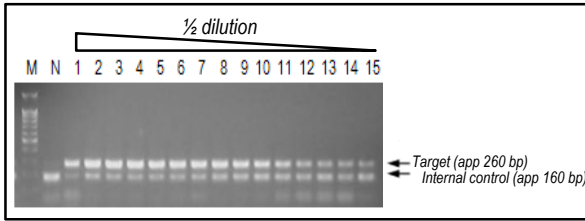


Fig. 4. Result of determining minimal required concentration of Mycoplasma per test

To determine minimal required concentration of Mycoplasma, *M. fermentans* was selected as a model Mycoplasma species. *M. fermentans* was serially diluted for PCR detection. The result indicates that the detection limit with this kit is 20 cfu/ml as a Mycoplasma concentration in culture broth.

Lane	M	N	1	2	3	4	5	6
Copy number	100 bp DNA Marker	0	3.4x10 ⁵	1.7x10 ⁵	8.25x10 ⁴	4.12x10 ⁴	2.06x10 ⁴	1.03x10 ⁴
Lane	7	8	9	10	11	12	13	14
Copy number	5.1x10 ³	2.5x10 ³	1.28x10 ³	6.4x10 ²	3.2x10 ²	161	80	40

SPECIES DETERMINATION BY SEQUENCING ANALYSIS

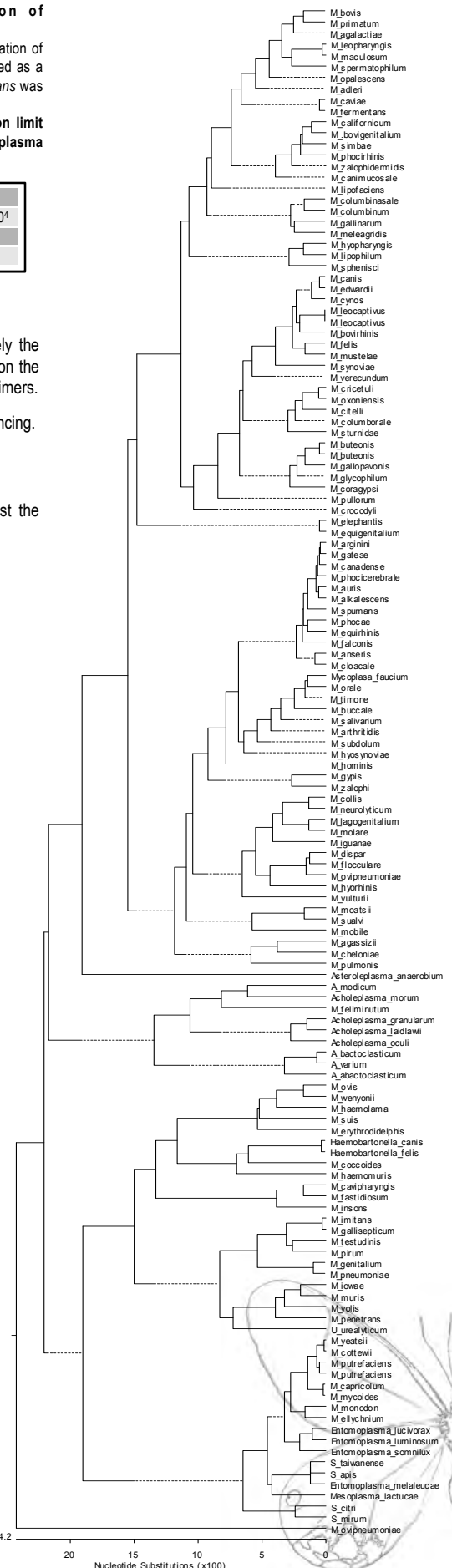
- The sequences of PCR products have slight differences among species. You can determine approximately the Mycoplasma species by sequencing analysis with the following primer. Please refer to the phylogenetic tree on the right side. For more detailed species analysis, you should perform additional sequencing with your designed primers.
- We provide only the Forward primer sequence. Please synthesize the primer, and then use it in general sequencing.
- Nucleotide sequence of the sequencing primer:
5' – GGA TTA GAT ACC CTG GTA GTC CAC G– 3'

[Note] The PCR primers set included in this kit differs from the above sequencing primer. We do not list the sequences of primers comprising the PCR primers set contained in this kit.

DETECTABLE MYCOPLASMA STRAINS (8 Genus / 209 Species)

Genus	Species				
Acholeplasma (5)	<i>Acholeplasma granularum</i>	<i>Acholeplasma laidlawii</i>	<i>Acholeplasma modicum</i>	<i>Acholeplasma morum</i>	<i>Acholeplasma oculi</i>
Anaeroplasm (3)	<i>Anaeroplasm abactoclasticum</i>	<i>Anaeroplasm baetoclasticum</i>	<i>Anaeroplasm varium</i>		
Asteroleplasma (1)	<i>Asteroleplasma anaerobium</i>				
Entomoplasma (5)	<i>Entomoplasma lucivorax</i>	<i>Entomoplasma luminosum</i>	<i>Entomoplasma melaleuciae</i>	<i>Entomoplasma somnifux</i>	<i>Entomoplasma ellychniae</i>
Mycoplasma (182)	<i>M. adleri</i> <i>M. alligatoris</i> <i>M. arginini</i> <i>M. bovis</i> <i>M. canadense</i> <i>M. hyopharyngis</i> <i>M. cloacale</i> <i>M. columbale</i> <i>M. crocodyli</i> <i>M. equigenitalium</i> <i>M. faucium</i> <i>M. flocculare</i> <i>M. gateae</i> <i>M. haemocanis</i> <i>Mycoplasma sp. Ms01</i> <i>M. hyopneumoniae (strain 7448)</i> <i>M. hyosyngiae</i> <i>M. leocapivivus</i> <i>M. microti</i> <i>M. muris</i> <i>M. mycoides subsp. mycoides SC</i> <i>M. ovipneumoniae</i> <i>M. phocirhinis</i> <i>M. pulmonis</i> <i>M. maculosum</i> <i>M. suis</i> <i>M. testudinum</i> <i>M. weynonii</i> <i>Mycoplasma sp. Z61</i> <i>Mycoplasma sp. T014</i> <i>Mycoplasma sp. Z371AT</i> <i>Mycoplasma sp. 8790CV</i> <i>Mycoplasma sp. C3T</i> <i>Mycoplasma sp. HRC689</i> <i>Mycoplasma sp. M209-8</i> <i>M. capricolum subsp. capripneumonii</i> <i>Mycoplasma sp. Saa1c</i>	<i>M. agalactiae (strain PG2)</i> <i>M. alvi</i> <i>M. arthritis</i> <i>M. bovovulvi</i> <i>M. canis</i> <i>M. coccoides</i> <i>M. conjunctivae</i> <i>M. cynos</i> <i>M. felis</i> <i>M. felifaucium</i> <i>M. gallinarum</i> <i>M. genitalium</i> <i>M. haemofelis</i> <i>Mycoplasma sp. Ms02</i> <i>M. insonis</i> <i>M. indense</i> <i>M. leoncapivi</i> <i>M. moatsii</i> <i>M. mustelae</i> <i>M. mycoides subsp. capri</i> <i>M. oxoniensis</i> <i>M. phurum</i> <i>M. putrefaciens</i> <i>M. subdolum</i> <i>M. testudinum</i> <i>M. yeatsii</i> <i>Mycoplasma sp. SF12</i> <i>Mycoplasma sp. T1CL2</i> <i>Mycoplasma sp. ZF1AT</i> <i>Mycoplasma sp. 94630</i> <i>Mycoplasma sp. China-1</i> <i>Mycoplasma sp. IS2505</i> <i>Mycoplasma sp. M221-9</i> <i>M. capricolum subsp. capripneumoniae</i> <i>Mycoplasma sp. SF9</i>	<i>M. amphoriforme</i> <i>M. auris</i> <i>M. buccale</i> <i>M. capricolum</i> <i>M. caviae</i> <i>M. collis</i> <i>M. corogypsi</i> <i>M. dispar</i> <i>M. erythrodelphis</i> <i>M. feliminum</i> <i>M. gallinarum</i> <i>M. genitalium G37</i> <i>M. haemolama</i> <i>Mycoplasma sp. Ms03</i> <i>M. hyorhinis</i> <i>M. iuganae</i> <i>M. lipofaciens</i> <i>M. molare</i> <i>M. mycoides</i> <i>M. neurolyticum</i> <i>M. oalescens</i> <i>M. penetrans</i> <i>M. pneumoniae</i> <i>M. salivarum</i> <i>M. sphenisci</i> <i>M. suis</i> <i>M. timone</i> <i>M. zolophi</i> <i>Mycoplasma sp. 07SH-h</i> <i>Mycoplasma sp. 1220</i> <i>Mycoplasma sp. 34CL</i> <i>Mycoplasma sp. A1802T</i> <i>Mycoplasma sp. CSL 4779</i> <i>Mycoplasma sp. M1</i> <i>Mycoplasma sp. M222-2</i> <i>Mycoplasma. sp.</i> <i>ovine/caprine serogroup 11</i>	<i>M. agassizii</i> <i>M. anatis</i> <i>M. bovirhinis</i> <i>M. californicum</i> <i>M. capricolum</i> <i>M. cavipharyngis</i> <i>M. columbinum</i> <i>M. cotti</i> <i>M. edwardii</i> <i>M. falconis</i> <i>M. felis</i> <i>M. gallisepticum</i> <i>M. gypsis</i> <i>M. hominis</i> <i>Mycoplasma sp. Ms04</i> <i>M. imitans</i> <i>M. iners</i> <i>M. lipophilum</i> <i>M. monodon</i> <i>M. mycoides subsp. capri</i> <i>M. orale</i> <i>M. phocicebrale</i> <i>M. primum</i> <i>M. simbae</i> <i>M. spumans</i> <i>M. sturni</i> <i>M. verecundum</i> <i>M. zolophidemidis</i> <i>Mycoplasma sp. 07SH-p</i> <i>Mycoplasma sp. 13CL</i> <i>Mycoplasma sp. 39CL</i> <i>Mycoplasma sp. ARNO</i> <i>Mycoplasma sp. CSL 7518-lung</i> <i>Mycoplasma sp. M200-2</i> <i>Mycoplasma sp. M222-5</i> <i>M. hyopneumoniae (strain J / ATCC 25934)</i>	<i>M. alkalescens</i> <i>M. anseris</i> <i>M. bovirhinis</i> <i>M. californicum</i> <i>M. capricolum subsp. capricolum</i> <i>M. citelli</i> <i>M. columbinum</i> <i>M. cricetuli</i> <i>M. elephantis</i> <i>M. fastidiosum</i> <i>M. fermentans</i> <i>M. gallopavonis</i> <i>M. gypsis</i> <i>M. hominis</i> <i>M. iuganae</i> <i>M. lipophilum</i> <i>M. monodon</i> <i>M. mycoides subsp. mycoides LC</i> <i>M. orale</i> <i>M. phocidae</i> <i>M. pullorum</i> <i>M. spermatoophilum</i> <i>A. modicum</i> <i>Acholeplasma_morum</i> <i>M. felinum</i> <i>Acholeplasma_granularum</i> <i>Acholeplasma_laidlawii</i> <i>Acholeplasma_oculi</i> <i>A. abactoclasticum</i> <i>A. varium</i> <i>A. abactoclasticum</i> <i>M. suis</i> <i>M. weynonii</i> <i>M. haemolama</i> <i>M. suis</i> <i>M. erythrodelphis</i> <i>Haemobartonella_caris</i> <i>Haemobartonella_felis</i> <i>M. coccoides</i> <i>M. haemomuris</i> <i>M. cavipharyngis</i> <i>M. fastidiosum</i> <i>M. iners</i> <i>M. imitans</i> <i>M. gallisepticum</i> <i>M. testudinum</i> <i>M. pneumoniae</i> <i>M. genitalium</i> <i>M. pneumoniae</i> <i>M. muris</i> <i>M. volis</i> <i>M. penetrans</i> <i>U. urealyticum</i> <i>M. yeatsii</i> <i>M. cotti</i> <i>M. putrefaciens</i> <i>M. putrefaciens</i> <i>M. capricolum</i> <i>M. mycoides</i> <i>M. mycoides</i> <i>Entomoplasma_lucivorax</i> <i>Entomoplasma_luminosum</i> <i>Entomoplasma_somnifux</i> <i>S. taiwanense</i> <i>S. apsis</i> <i>Entomoplasma_melaleuciae</i> <i>Mesoplasma_lactucaee</i> <i>S. citri</i> <i>S. mirum</i> <i>M. ovipneumoniae</i>
Mesoplasma (3)	<i>Mesoplasma entomophilum</i>	<i>Mesoplasma florum</i>	<i>Mesoplasma lactucaee</i>		
Spiroplasma (9)	<i>Spiroplasma apis</i>	<i>Spiroplasma citri</i>	<i>Spiroplasma CN-5</i>	<i>Spiroplasma DU-1</i>	<i>Spiroplasma DW-1</i>
Ureaplasma (1)	<i>Ureaplasma urealyticum</i>				

PHYLOGENETIC ANALYSIS



PARTIAL SEQUENCES OF MAJOR CONTAMINANTS IN CELL CULTURE

The following sequences are partial sequences of major contaminant in general cell culture or pathogenic Mycoplasma strains. You can determine the species by sequencing analysis.

