e-Myco™ Mycoplasma PCR Detection Kit (ver. 2.0, for 20 μl rxn)

Cat. No. 25233 96 Tests

DESCRIPTION

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. Because the presence of contaminant mycoplasma can be easily detected by only verifying the bands of amplified DNA fragments using electrophoresis, there is no need to prepare probes that are labeled with radioisotope, etc. You can determine the species groups of mycoplasma by sequencing analysis using the sequencing primers suggested in this manual. 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*-StarTaq™ 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.

KIT CONTENTS and STORAGE

- *e*-Myco™ Mycoplasma PCR Detection Kit (ver.2.0) — 96 tubes
- DNase/RNase-free Distilled Water — 1 vial (1 ml)
- Store at -20°C. The *e*-Myco™ Mycoplasma Detection PCR Kit (ver.2.0) is a novell vacuum-dried premix type. We guarantee storage for 12 months at -20°C.

COMPONENT

- PCR Reaction volume — 20 μl reaction

e-Myco™ Mycoplasma PCR Detection Kit (ver. 2.0)	
i-StarTaq™ 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 Primer Sets	10 pmol / each
Internal Control	
8-MOP (dissolved in DMSO)	

Dried under iNtRON's instruction (Patent Pending)

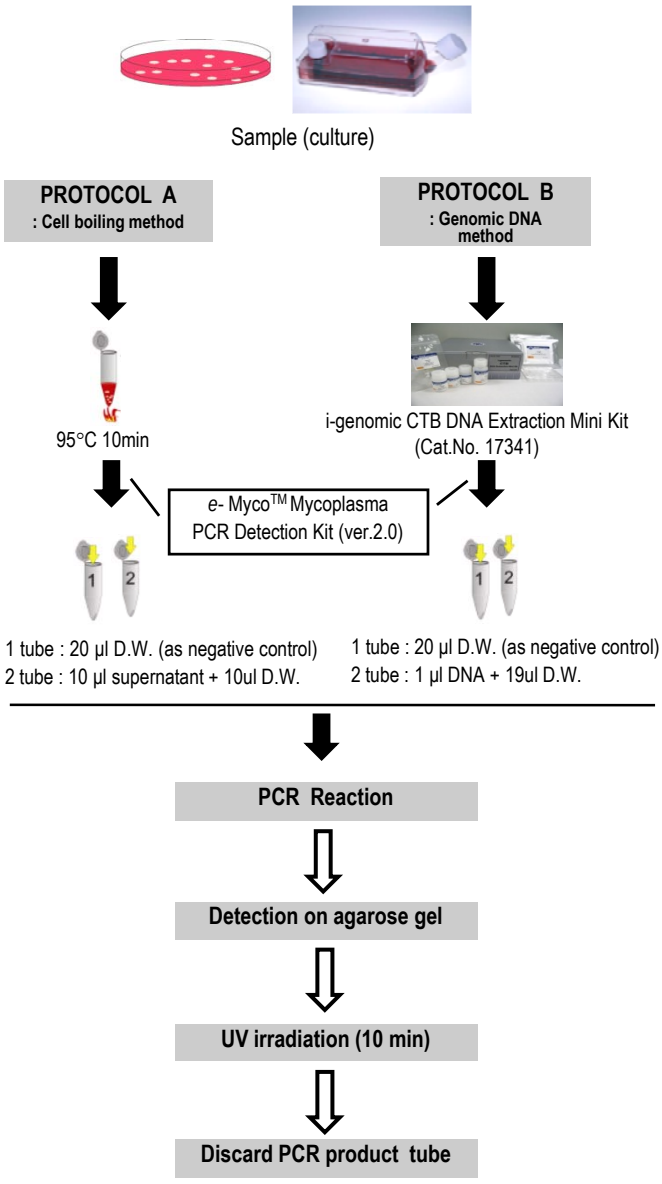
CHARACTERISTICS

- **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 (See Technical Guide).
- **Species Determination**
  - You can determine the species of mycoplasma by sequencing the amplified PCR products.
- **Internal Control**
  - Internal 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.

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, this kit can detect other various species of mycoplasma (See Technical Guide).

[Overview of Mycoplasma Detection]



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). 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 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.
- 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-spin™ Agarose Gel DNA Exttaction, iNtRON , Cat.No 17181, MEGAquick-spin™ PCR & Agarose Gel DNA Extraction Kit, iNtRON , Cat.No 17281)

• PROTOCOL A : Using the Boiling Extract 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 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 5x10^4 cells per test (see Technical Guide, >50,000 cells are needed to complete this protocol).

   Note 2: Strong mycoplasma infections are detected in as 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.

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 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.**
8. **Add 10 µl of the template to each tube of *e*-Myco™ Mycoplasma PCR Detection Kit (ver. 2.0), and then resuspend after adding 10 µl of sterile water for a 20-µl PCR reaction volume.**
9. **Perform PCR reaction as in the following table.**
   Note : We recommend that you perform one negative control reaction by adding 20 µl of sterile water.

PCR Condition		Temp.	Time
Initial denaturation		94°C	1 min
35 cycles	Denaturation	94°C	30 sec
	Annealing	60°C	20 sec
	Extension	72°C	1 min
Final extension		72°C	5 min

10. **For analysis by electrophoresis, use 5 µl of each tube.**
11. **PCR products should be discarded after UV irradiation (10 min) to prevent carry-over contamination.**

    Note: Contamination of DNA is a serious problem of PCR. Please discard PCR products after UV irradiation (365 nm) to prevent carry-over contamination.

• PROTOCOL B : Using genomic DNA as a template

1. **Add purified genomic DNA as a template using the i-genomic CTB DNA Extraction Mini Kit (Cat.No. 17431) to each tube of *e*-Myco™ Mycoplasma PCR Detection Kit (ver. 2.0), and then resuspend after adding sterile water for a 20-µl PCR reaction volume**
   Note: Appropriate amounts of DNA template sample: genomic DNA, 50 ng–100 ng
2. **Follow protocol A from step 9.**
   Note: We recommend that you perform one negative control reaction by adding 20 µl of sterile water.

TROUBLESSHOOTING GUIDE

1. No band in positive sample

- Check Internal control band
  : If internal control band is seen, PCR has been performed properly; it is not a problem of the product.
- Check template quality
  :Even though DNA is isolated from the sample, the PCR reaction can be inhibited depending on DNA purity in some cases. In this case, extracted DNA should be diluted 10 times with D.W. and used to perform PCR again.
- Check 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 high template concentration. Please proceed with a lower concentration of DNA.
- Check template quality
  : Even though DNA is isolated from the sample, the PCR reaction can be inhibited depending on DNA purity in some cases. In this case, extracted DNA should be diluted 10 times with D.W. and used to run the PCR reaction again. If still no band is seen, please inquire with our technical support staff.

3. Amplicon bands 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. We recommend that you use a pipette after sterilization. Proceed with all procedures on a clean bench and keep the location where your procedures are performed sterile.

4. Poor resolution on agarose gel

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

TECHNICAL GUIDE

PRINCIPLE OF MYCOPLASMA DETECTION

- The newly developed *e*-Myco™ Mycoplasma PCR Detection Kit (ver.2.0) is a highly sensitive PCR assay that detects various *Mycoplasma* species that may contaminate cell culture samples. The primer sets primarily allow for detection of major mycoplasma species in cell culture contaminations (*M. arginini, M. faucium, M. fermentans, M. hyorhinis, M. orale*) as well as *Acholeplasma laidlawii*. Furthermore, you can detect various mycoplasma species with this kit (see below). It is a quick, simple, reliable, and cost-effective method for regularly monitoring cells for mycoplasma detection.

- The primer sets used in the kit are derived from a highly conserved region within the 16S rRNA gene and can detect very low levels of contamination. The rRNA gene sequences of prokaryotes, including mycoplasma, are well conserved, whereas the lengths and sequences of the spacer region in the rRNA operon differ from species to species. So, you can determine the species by sequencing analysis.

ANALYTIC INFORMAION

◆ *Origin Type*

Table 1 shows the broad species of mycoplasma detected by this kit. As shown, this kit can detect a broad range of mycoplasma with high specificity and sensitivity. The name Mycoplasma comes from the Greek words mykes (fungus) and plasma (formed) and was proposed in the 1950s. Mycoplasma is a genus of small bacteria that lack cell walls. Many species were purified and characterized from various origins such as cell culture, human, and cows. We classify them briefly by origin.

Type	Origin	Type	Origin
A	Cell culture	L	Guinea Pig
B	Human	M	Squirrels
C	Avian	N	Turkey
D	Porcine	O	Puma
E	Bovine	P	Canine
F	Ovine	Q	Primates
G	Equine	R	Lion
H	Murine	S	Monkey
I	Insect	T	Mink
J	Goat	U	Hamster
K	Geese	V	Iguana

◆ *Target Primers*

The target regions in this kit are divided into seven M types (M1~M7) and one A type for detecting the bulk of the species in the genus Mycoplasma. The designed primers are sufficient to detect major contaminants in cell cultures such as *M. arginini, M. faucium, M. fermentans, M. hyorhinis, M. orale,* and *A. laidlawii* as well as other broad species of mycoplasma.

Type	Designed Primer*
M1	Standard
M2	15C→T
M3	16T→C
M4	17C→T
M5	18T→C
M6	18T→C, 20T→A
M7	8T→C
A	*A. laidlawii* only

*Not revealed primer sequences

◆ *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.

Type	PCR Size
I	268 bp
II	269 bp
III	270 bp
VI	271 bp
V	272 bp
VI	277 bp

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 :
  M. species Forward 5'- GAT TAG ATA CCC TGG TAG TC-3' (20 mer)
  A. laidlawii Forward 5'- GAT ACC CTG GTA GTC CAC GC-3' (20 mer)

[Note] The PCR primers used in this kit differ from the sequencing primers. We do not list the PCR primer sequences contained in this kit.

PARTIAL SEQUENCES OF MAJOR CONTAMINANTS IN CELL CULTURE

The following sequences are partial sequences of major contaminants in general cell culture. You can classify the species by sequencing analysis.

```
                    *        20         *        40         *
M. arginini :  GATTAGATACCCTGGTAGTCCACGCCGTAAACGATGATCATTAGTGGTGGA :  52
M. faucium :   GATTAGATACCCTGGTAGTCCACGCCGTAAACGATGATCATTAGTGGTGGG :  52
M. fermentans : GATTAGATACCCTGGTAGTCCACGCTGTAAACGATGATCATTAGTGGTGGA :  52
M. hyorhinis : GATTAGATACCCTGGTAGTCCACGCCGTAAACGATGATCATTAGTTGGTGGA :  52
M. orale :     GATTAGATACCCTGGTAGTCCACGCCGTAAACGATGATCATTAGCTGATGGG :  52
A. laidlawii : -----GATACCCTGGTAGTCCACGCCGTAAACGATGAGAACTAAGTGTTGGC :  47
               gattaGATACCCTGGTAGTCCACGCcgTAAACGATGAtcAtTAg  G TGG

                 60         *        80         *        100
M. arginini : -G--AGTTCACTGACGCAGCTAACGCATTAAATGATCCGCCTGAGTAGTATG : 101
M. faucium :  -A--G--CCACTGACGCAGCTAACGCATTAAATGATCCGCCTGAGTAGTATG :  99
M. fermentans : -A--AA-CTACTGACGCAGCTAACGCATTAAATGATCCGCCTGAGTAGTATG : 100
M. hyorhinis : -ATAATTTCACTAACGCAGCTAACGCGTTAAATGATCCGCCTGAATAGTATG : 103
M. orale :     -G--AACTCATCGCGCAGCTAACGCATTAAATGATCCGCCTGAGTAGTACG : 101
A. laidlawii : CAAAAGGTCACTGCTGCAGTAACGCATTAAGTTCTCCGCCTGAGTAGTACG :  99
                   a   cA tg cGCAGcTAACGCaTTAAaTgaTCCGCCTGAgTAGTA G

                  *       120         *       140         *
M. arginini : CTCGCAAGAGTGAAACTTAAAGGAATTGACGGGGACCCGCACAAGCGGTGGA : 153
M. faucium :  CTCGCAAGAGTGAAACTTAAAGGAATTGACGGGGACCCGCACAAGCGGTGGA : 151
M. fermentans : CTCGCAAGAGTGAAACTTAAAGGAATTGACGGGGACCCGCACAAGCGGTGGA : 152
M. hyorhinis : CTCGCAAGAGTGAAACTTAAAGGAATTGACGGGAACCCGCACAAGCGGTGGA : 155
M. orale :     TTCGCAAGAATAAAACTTAAAGGAATTGACGGGGATCCGCACAAGCGGTGGA : 153
A. laidlawii : TACGCAAGTATGAAACTCAAAGGAATTGACGGGACCCCGCACAAGCGGTGGA : 151
                tCGCAAGa TgAAACTtAAAGGAATTGACGGG acCCGCACAAGCGGTGGA

                160         *       180         *       200
M. arginini : GCATGTGGTTTAATTTGAAGATACGCGGAGAACCTTACCCACTCTTGACATC : 205
M. faucium :  GCATGTGGTTTAATTTGAAGATACGCGGAGAACCTTACCCACTCTTGACATC : 203
M. fermentans : GCATGTGGTTTAATTTGAAGATACGCGGAGAACCTTACCCACTCTTGACATC : 204
M. hyorhinis : GCATGTGGTTTAATTTGAAGATACGCGTAGAACCTTACCCACTCTTGACATC : 207
M. orale :     GCATGTGGTTTAATTTGAAGATACGCGTAGAACCTTACCCACTCTTGACATC : 205
A. laidlawii : TCATGTTGTTTAATTCGAAGATACACGAAAAACCTTACCAGGTCTTGACATA : 203
               gCATGTgGTTTAATTtGAAGATACgCG AgAACCTTACCcacTCTTGACATc

                *       220
M. arginini : CTTGGCAATGCTATA-- : 220
M. faucium :  CTTTGCAAAGCTATAGA : 220
M. fermentans : CCCTGCAAAGCTATAG- : 220
M. hyorhinis : TTCTGCAAAGCTA---- : 220
M. orale :     TTCTGCAAAGCTATG-- : 220
A. laidlawii : CTCTGCAAAGTTCGGAG : 220
                t tGCAAaGcTa
```

These sequences are the partial sequences of PCR products

Table 1. Mycoplasma Species Detected by *e*-Myco™ Kit (ver.2.0)

M. species	*Origin Type*	*Primer Type*	*PCR Size*
A. laidlawii	A/E	A	I
M. adleri	J	M2	III
M. agalactiae	F	M2	III
M. alkalescenns	E	M5	III
M. anseris	K	M5	II
M. arginini	A/B	M5	III
M. arthritidis	H	M5	III
M. auris	F	M5	III
M. bovigenitalium	E	M2	III
M. bovirhinis	E	M2	IV
M. bovis	E	M2	III
M. buccale	B	M5	II
M. californicum	E	M2	II
M. canadense	E	M5	III
M. caviae	L	M2	III
M. citelli	M	M2	I
M. cloacale	N	M5	II

M. species	*Origin Type*	*Primer Type*	*PCR Size*
M. columbinasale	C	M2	II
M. columbinum	C	M2	III
M. equirhinis	G	M4	II
M. falconis	C	M2	IV
M. faucium	A	M1	I
M. felifaucium	O	M2	II
M. fermentans	A	M2	III
M. gallinarum	C	M2	III
M. gateae	P	M5	III
M. hominis	A	M6	II
M. hyorhinis	A/D	M2	V
M. hyosynoviae	P	M5	II
M. iguanae	V	M4	III
M. indiense	Q	M3	II
M. iners	C	M2	II
M. leopharyngis	R	M2	II
M. maculosum	P	M2	II

M. species	*Origin Type*	*Primer Type*	*PCR Size*
M. meleagridis	C	M2	II
M. moatsii	S	M2	III
M. mustelae	T	M2	I
M. opalescens	P	M2	III
M. orale	A	M3	II
M. oxoniensis	U	M2	I
M. penetrans	B	M7	VI
M. primatum	Q	M2	III
M. pulmonis	H	M5	IV
M. salivarium	A	M5	II
M. spermatophilum	B	M2	II
M. sualvi	P	M2	III
M. subdolum	G	M5	III
M. synoviae	C	M2	I
M. verecundum	E	M2	I

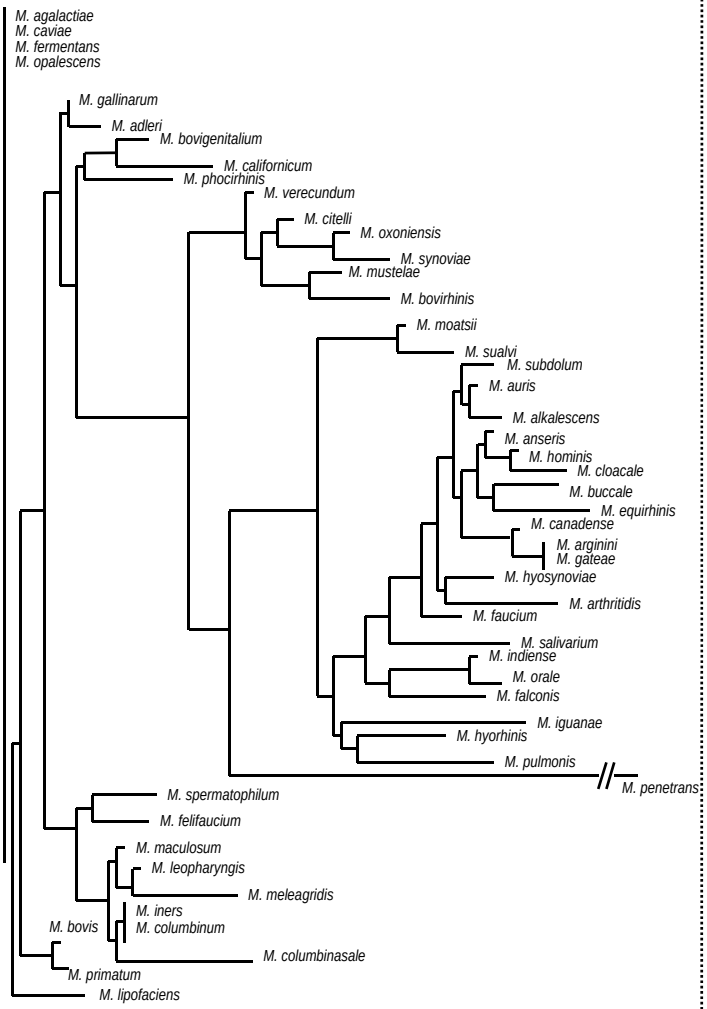
TECHNICAL GUIDE

TECHNICAL INFORMATION

- This e-Myco™ Mycoplasma PCR Detection Kit (ver.2.0) will provide 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 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 i-genomic CTB DNA Extraction Mini Kit (Cat. No. 17341).

PHYLOGENETIC ANALYSIS TABLE

- The following phylogenetic analysis table shows the classification based on the sequence variations of PCR-amplified products. This cluster can be changed by which sequences are based on. This cluster is just a reference table.
- With a suggested sequencing primer, you can approximately determine the species. For example, because the cluster between *M. fermentans* and *M. gallinarum* is different, you can simply classify the species with just sequencing analysis. However, there is no difference between *M. agalactiae*, *M. caviae*, *M. fermentans*, and *M. opalescens*. In this case, you can't determine the detailed species with only this kit and a suggested sequencing primer. If you want to know the detailed species, you have to synthesize your own PCR primers, and then analyze by sequencing analysis.



EXPERIMENTAL INFORMATION

◆ *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

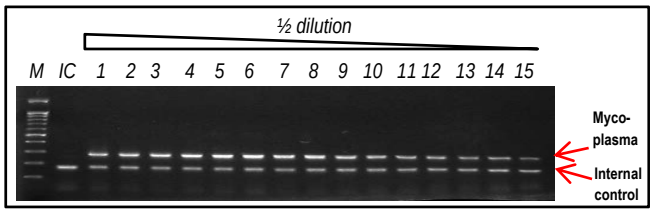


Fig.1. Mycoplasma detection was performed for genomic DNA
Genomic DNA was isolated from *M. fermentans*-infected K562 using the i-genomic CTB DNA Extraction Mini Kit (17341). The isolated gDNA was serially diluted for PCR of mycoplasma detection. **These results show that it can be applied to mycoplasma detection with small quantities, such as 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

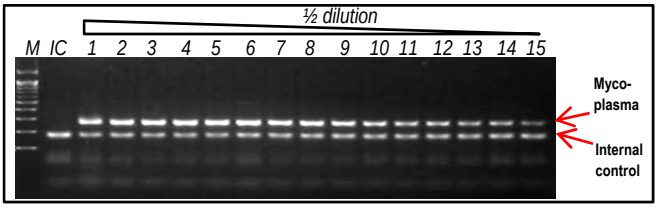


Fig.2. Mycoplasma detection was performed using the *e*-Myco™ Mycoplasma PCR Detection Kit (ver.2.0) method
Mycoplasma detection from cell lysates of *M. fermentans*-infected K562 using the *e*-Myco™ Mycoplasma Detection Kit (ver.2.0). The *M. fermentans*-infected K562 cells were serially diluted for 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

3) Result for Mycoplasma copy number

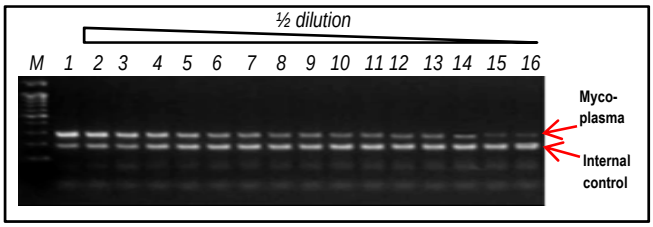
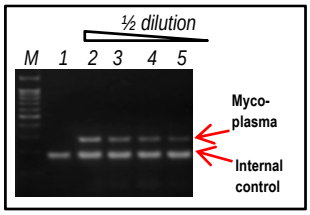


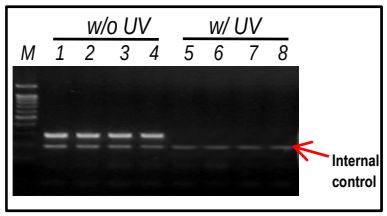
Fig.3. Mycoplasma detection was performed using the *e*-Myco™ Mycoplasma PCR Detection Kit (ver.2.0) method
Mycoplasma detection from *fermentans using the e*-Myco™ Mycoplasma Detection Kit. *M. fermentans* were serially diluted for PCR of mycoplasma detection. **These results show that it can be applied to mycoplasma detection with small copy numbers, such as 20 cfu/ml**
Lane M, 100bp DNA Marker; **lane1**, $6.6x10^5$; **lane 2**, $3.3x10^5$; **lane 3**, $1.65x10^5$; **lane 4**, $8.25x10^4$; **lane 5**, $4.12x10^4$; **lane 6**, $2.06x10^4$; **lane 7**, $1.0x10^4$ **lane 8**, $5.1x10^3$; **lane 9**, $2.5x10^3$; **lane 10**, $1.28x10^3$; **lane 11**, $6.4x10^2$; **lane 12**, $3.22x10^2$ **lane13**, $1.61x10^2$; **lane 14**, 80; **lane 15**, 40; **lane 16**, 20

◆ *Elimination of Carryover Contamination*



1) 1st PCR

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

TECHNICAL GUIDE

PARTIAL SEQUENCES OF MYCOPLASMA SPECIES AMPLIFIED BY e-Myco™Mycoplasma Detection Kit (ver.2.0)

> *A. laidlawii*
GATACCCTGGTAGTCCACGCCGTAAACGATGAGAACTAAGTGTTGGCCAAAAGGT
CAGTGCTGCAGTTAACGCATTAAGTTCTCCGCCTGAGTAGTACGTACGCAAGTAT
GAAACTCAAAGGAATTGACGGGACCCCGCACAAGCGGTGGATCATGTTGTTTAAT
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> *M. adleri*
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> *M. agalactiae*
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> *M. alkalescens*
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> *M. anseris*
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> *M. arginini*
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> *M. arthritidis*
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> *M. auris*
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> *M. bovigenitalium*
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> *M. bovirhinis*
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> *M. bovis*
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> *M. buccale*
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> *M. californicum*
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> *M. canadense*
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> *M. caviae*
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> *M. citelli*
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> *M. cloacale*
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> *M. columbinasale*
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> *M. columbinum*
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> *M. equirhinis*
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> *M. falconis*
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> *M. faucium*
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> *M. felifaucium*
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> *M. fermentans*
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>*M. gallinarum*
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> *M. gateae*
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> *M. hominis*
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> *M. hyorhinis*
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> *M. hyosynoviae*
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>*M. iguanae*
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> *M. indiense*
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> *M. iners*
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> *M. leopharyngis*
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> *M. maculosum*
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> *M. meleagridis*
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> *M. moatsii*
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> *M. mustelae*
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> *M. opalescens*
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> *M. orale*
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> *M. oxoniensis*
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> *M. penetrans*
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> *M. primatum*
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> *M. pulmonis*
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> *M. salivarium*
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> *M. spermatophilum*
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> *M. sualvi*
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> *M. subdolum*
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> *M. synoviae*
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> *M. verecundum*
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