Test for the detection of Mycoplasmas by PCR analysis

e-Myco™ VALiD
Mycoplasma PCR Detection Kit

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RUO *Research Use Only* REF *25239* Σ 48 *25240* Σ 8

DESCRIPTION

- The maintenance of contamination-free cell lines is essential to cell-based research such as biopharmaceutical production, cell therapy and tissue engineering. Mycoplasma is often not visible and does not respond to antibiotics, and therefore it is a major issue that requires monitoring and early detection. Up to 30~85% of cell cultures may be contaminated with mycoplasmas, the main contaminants being the species *M. orale, A. laidlawii, M. arginini* and *M. hyorhinis.* Although these mycoplasmas do not usually kill contaminated cells, they are difficult to detect and can cause a variety of effects on cultured cells, including changes in metabolism growth, viability and morphology, thereby altering the phenotypic properties of the host cells. Traditional detection methods use direct culture method to detect contaminating organisms. But the culture-based methods are time-consuming, requiring as much as 28 days, very laborious and difficult to interpret. Thus recently, it is a trend that PCR-based detection method may be adopted to standard protocol replacing direct culture method including E.P. 2.6.7 directive and drug regulating agencies worldwide.
- The e-Myco™ VALiD Mycoplasma PCR Detection Kit is composed a set of primers those are specific for the highly conserved mycoplasma 16S-rRNA coding region including *M. pneumoniae, M. agninini, M. hyorhinis, M. fermentans, M. orale* and *A. laidlawii*. The kit is design to detect the presence of mycoplasma that might contaminate biological materials such as cultured cells. Also, the kit can be performed in 3 hours, can detect sensitively until 10 CFU/ml and includes internal control for verifying a PCR run as well as positive control DNA. The adopted 8-methoxypsoralen (8-MOP) is used to extinguish the template activity of contaminated DNAs (PCR product). 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.
- Each tube of the e-Myco™ VALiD Mycoplasma PCR Detection Kit provides all-in-one system(FastMix technology), which means all components for PCR is already pre-aliquoted in each PCR tube. All you need to do is just to add template and distilled water for PCR.

CHACTACTERISTICS

- **Simple to Use :** This e-Myco™ VALiD Mycoplasma PCR Detection Kit contains all the components for the PCR reaction. You just add template DNA or samples.
- **Speed - 3 hours :** Replace traditional 28 days culture testing with the kit within 3 hours
- **Smart - internal control and 8-MOP :** Internal control system embedded in the product prevents misjudgment that possibly arises from an erroneous PCR test. And the kit can eliminate carry-over contamination with 8-MOP activation.
- **Steady - Broad Species Detection :** You can detect common cell culture-infecting species of mycoplasma and also other various species of mycoplasma (See Technical Guide).
- **Stage-up - Sensitive and reliable :** Highly sensitive PCR test for the detection of Mycoplasmas al least 10 CFU/ml, tested for validation according to the KFDA testing guidance similar with E.P. 2.6.7 directive (See Experimental Information). This test is suitable for release testing and in-process control. It can replace culture and indicator cell tests.

KIT CONTENTS

Label	Contain
e-Myco™ VALiD Mycoplasma PCR Premix	48T / 8 T
Control DNA (Recombinant DNA included partial 16S sequence of M. hyorhinis)	20 µl / 5 µl
DNase/RNase-free Distilled Water	1 ml / 0.2 ml

STORAGE AND STABILITY

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

APPLICATIONS

The kit is used for the detection of mycoplasma species that are most commonly encountered in cell culture, including *M. peumoniae, M. arginini, M. fermentans, M. hyorhinis, M. orale*, and *Acholeplasma laidlawii.* Furthermore, this kit can detect other various species of mycoplasma (See Technical Guide).

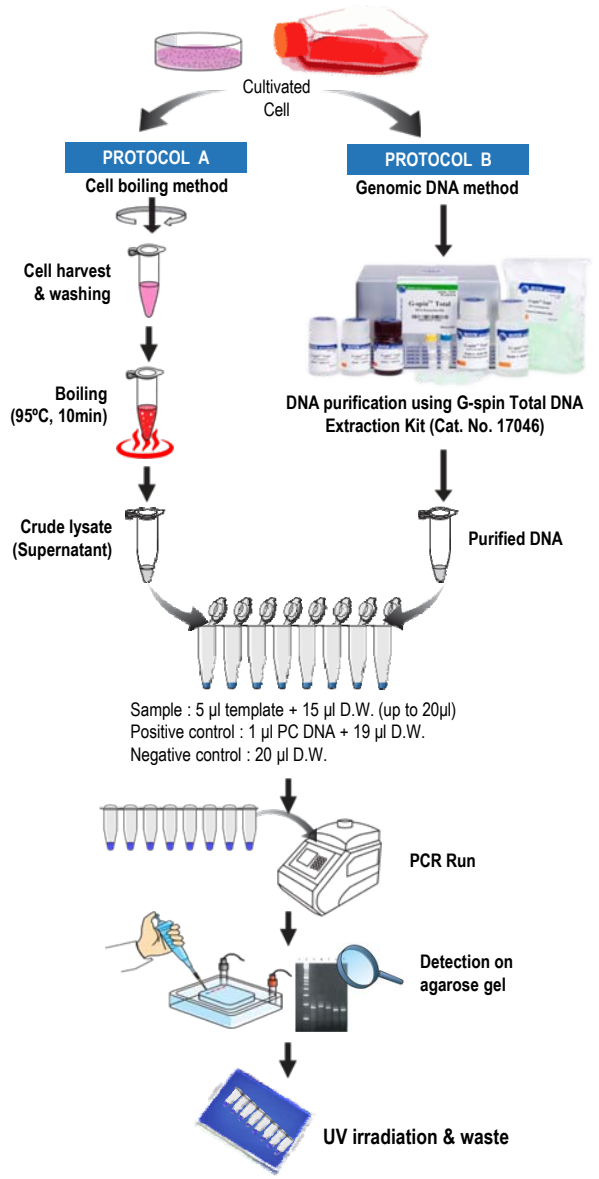
ADDITIONAL REQUIRED EQUIPMENT

- Distilled water
- Primers
- Pipettes and pipette tips (aerosol resistant)
- Thermal cycler
- Mineral oil (only if the thermal cycler does not have a heated lid)

NOTICE BEFORE USE

e-Myco™ VALiD Mycoplasma PCR Detection Kit is intended for research use only. Prior to using it for other purposes, the user must validate the system in compliance with the applicable law, directives, and regulations. e-Myco™ VALiD 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.

OVERVIEW OF MYCOPLASMA DETECTION



REACTION TUBE COMPONENT

• PCR Reaction volume 20 µL reaction

e-Myco™ VALiD Mycoplasma PCR Detection Kit

DNA Polymerase	2.5U	KCl	50 mM
Chemical Stabilizer	1X	$MgCl_2$	1.5 mM
Loading Buffer	1X	Mycoplasma Primers Set	
dNTPs	250mM each	Internal Control	
Tris-HCl (pH 8.3)	10 mM	8-MOP (dissolved in DMSO)	

Dried under iNtRON's instruction

PROTOCOLS

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. 17046). 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. MEGAquick-spin™ Total Fragment DNA Purification Kit, iNtRON , Cat.No 17286 ~ 17288)

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.
   Note 2: 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 ºC 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 5 µl of the template to each tube of e-Myco™ VALiD Mycoplasma PCR Premix, and then resuspend after adding 15 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 ºC	1 min
40 cycles	Denaturation	94 ºC	30 sec
	Annealing	60 ºC	20 sec
	Extension	72 ºC	1 min
Final extension		72 ºC	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 5 µl of purified genomic DNA as a template using the i-genomic CTB DNA Extraction Mini Kit (Cat.No. 17431) to each tube of G-spin Total DNA Extraction Kit (Cat. No. 17046), 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: Recommend to perform one negative control reaction by adding 20 µl of sterile water. We recommend to add 1 ~ 5 µl of control DNA for positive control reaction

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.

TECHNICAL INFORMATION

- This e-Myco™ VALiD Mycoplasma PCR Detection Kit 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.
- 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 G-spin Total DNA Extraction Kit (Cat. No. 17046).

PRINCIPLE OF MYCOPLASMA DETECTION

- The newly developed e-Myco™ VALiD Mycoplasma PCR Detection Kit 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. pneumoniae, 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 of Mycoplasma came 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	J	Rodents
B	Human	K	Canine
C	Primates	L	Puma
D	Bovine	M	Lion
E	Porcine	N	Sea lion
F	Avian	O	Elephant
G	Ovine	P	Mink
H	Goat	Q	Iguana
I	Equine	R	Insect

◆ 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	264 ~ 266 bp
II	268 bp
III	269 bp
IV	270 bp
V	271 bp
VI	272 bp
VII	276 bp
VIII	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 : AGGAT TAG ATA CCC TGG TAG TC-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.

```
M. arginini          1 AGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGATCATTAGTCGG
M. fermentans        1 AGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGATCATTAGCTGA
M. hyorhinis         1 AGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGATCATTAGTTGG
M. orale             1 AGGATTAGATACCCTGGTAGTCCACGCTGTAAACGATGATCATTAGTCGG
M. pneumoniae        1 AGGATTAGATACCCTAGTAGTCCACACCGTAAACGATAGATACTAGCTGT
A. laidlawii         1 AGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGAACTAAGTGT

M. arginini         51 TGGAG-----AGTTCACTGACGCAGCTAACGCATTAAATGATCCGCCTGA
M. fermentans       51 TGGGG-----AACTCATCGGCGCAGCTAACGCATTAAATGATCCGCCTGA
M. hyorhinis        51 TGGAATA---ATTTCACTAACGCAGCTAACGCGTTAAATGATCCGCCTGA
M. orale            51 TGGAA-----AACT-ACTGACGCAGCTAACGCATTAAATGATCCGCCTGA
M. pneumoniae       51 CGGGGCGATCCCCTCGGTAGTGAAGTTAACACATTAAGTATCTCGCCTGG
A. laidlawii        51 TGGGCAAA--AGGTCAGTGCTGCAGTTAACGCATTAAGTTCTCCGCCTGA

M. arginini        101 GTAGTATGCTCGCAAGAGTGAAACTTAAA-GGAATTGACGGGGACCCGCA
M. fermentans      101 GTAGTACGTTCGCAAGAATAAAACTTAAA-GGAATTGACGGGGATCCGCA
M. hyorhinis       101 GTAGTATGCTCGCAAGAGTGAAACTTAAA-GGAATTGACGGGAACCCGCA
M. orale           101 GTAGTATGCTCGCAAGAGTGAAACTTAAA-GGAATTGNNGGGGANNCGCA
M. pneumoniae      101 GTAGTACATTCGCAAGAATGAAACTCAAACGGAATTGACGGGGACCCGCA
A. laidlawii       101 GTAGTACGTACGCAAGTATGAAACTCAAA-GGAATTGACGGGACCCCGCA

M. arginini        151 CAAGCGGTGG-AGCATGTGG
M. fermentans      151 CAAGCGGTGG-AGCATGTGG
M. hyorhinis       151 CAAGCGGTGG-AGCATGTGG
M. orale           151 CAAGCGGTGG-AGCNTGTGG
M. pneumoniae      151 CAAGTGGTGG-AGCATGTTG
A. laidlawii       151 CAAGCGGTGGGATCATGTTG
```

This sequences are the partial sequence of PCR products

Table 1. Mycoplasma Species Detected by e-Myco™ VALiD Mycoplasma PCR Detection Kit

Mycoplasmas	*Origin Type*	*PCR Size*	*Mycoplasmas*	*Origin Type*	*PCR Size*	*Mycoplasmas*	*Origin Type*	*PCR Size*	*Mycoplasmas*	*Origin Type*	*PCR Size*
A. granularum	E	I	*M. canadense*	D	270	*M. hominis*	A	III	*M. oxoniensis*	J	II
A. laidlawii	A/D	II	*M. caviae*	J	270	*M. hyopharyngis*	E	270	*M. penetrans*	B	277
A. oculi	G	I	*M. citelli*	J	II	*M. hyorhinis*	A/E	272	*M. phocirhinis*	N	270
M, gallopavonis	F	III	*M. cloacale*	F	III	*M. Hyosynoviae*	K	III	*M. pirum*	B	276
M. adleri	H	270	*M. columbinasale*	F	III	*M. Iguanae*	Q	270	*M. pneumoniae*	B	276
M. agalactiae	G	270	*M. columbinum*	F	270	*M. Indiense*	C	III	*M. primatum*	C	270
M. alkalescenns	D	270	*M. cricetuli*	J	II	*M. Iners*	F	III	*M. pulmonis*	J	271
M. alvi	D	276	*M. elephantis*	O	270	*M. Iowae*	F	277	*M. salivarium*	A	III
M. anseris	F	III	*M. equigenitalium*	I	270	*M. Leocaptivus*	M	II	*M. simbae*	M	270
M. arginini	A/B	270	*M. equirhinis*	I	III	*M. Leopharyngis*	M	III	*M. spermatophilum*	B	III
M. arthritidis	J	270	*M. falconis*	F	271	*M. Lipofaciens*	F	270	*M. sphenisci*	F	272
M. auris	G	270	*M. faucium*	A	II	*M. Lipophilum*	B	270	*M. spumans*	K	270
M. bovigenitalium	D	270	*M. felifaucium*	L	III	*M. maculosum*	K	III	*M. sualvi*	K	270
M. bovirhinis	D	271	*M. fermentans*	A	270	*M. Meleagridis*	F	III	*M. subdolum*	I	270
M. bovis	D	270	*M. gallinarum*	F	270	*M. Moatsii*	C	270	*M. synoviae*	F	II
M. buccale	B	III	*M. gateae*	K	270	*M. mustelae*	P	II	*M. verecundum*	D	II
M. buteonis	F	II	*M. glycophilum*	F	II	*M. opalescens*	K	270	*M. zalophi*	N	270
M. californicum	D	III	*M. gypis*	F	270	*M. orale*	A	III	*M. zalophidermidis*	N	270

EXPERIMENTAL INFORMATION

◆ Interpretation

e-Myco™ VALiD Mycoplasma PCR Detection Kit provides the detection of various Mycoplasma species with high sensitivity and specificity. For the validation of each PCR reaction, it includes internal control in each PCR Premix tube. The interpretation of experimental results is as follows.

Band location : Mycoplasma – 260 ~ 280 bp, Internal control – 170 bp

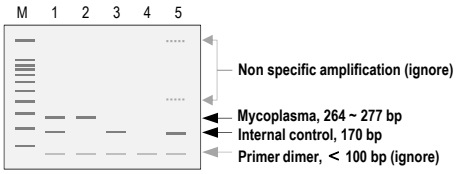


Fig. 1. Results Interpretation of e-Myco™ VALiD Mycoplasma PCR Detection Kit – Exemplary

Lane	1	2	3	4	5
Mycoplasma	Positive	Positive	Negative	Unknown	Negative
Results	Valid	Valid (a lot of target DNA amount)	Valid (Negative, below 10 cfu/ml)	Invalid, Retest (Poor reaction condition or low quality of template DNA)	Valid (low quality of template DNA or template degradation, ignore the non specific bands or primer dimer band)

◆ Analytical Sensitivity

e-Myco™ VALiD Mycoplasma PCR Detection Kit is an eligible kit for the efficient detection of *Mycoplasma spp.* contamination with high sensitivity in the culture.
To identify the analytical sensitivity, the genomic DNA from cell cultured 6 Mycoplasma spp. were purified. Mycoplasma species used for this experiment are as follows.

[Mycoplasma sample description]

Lane	Name	ATCC No.	Genome Size
1	*Mycoplasma orale*	15539	0.71 Mbp
2	*Mycoplasma hyorhinis*	17981	0.84 Mbp
3	*Mycoplasma fermentans*	15474	1.00 Mbp
4	*Mycoplasma pneumoniae*	15293	0.82 Mbp
5	*Mycoplasma arginini*	23243	0.62 Mbp
8	*Acholeplasma laidlawii*	14089	1.50 Mbp

The sensitivity according to the DNA copy number was investigated after purifying gDNA from each cultured Mycoplasma spp.

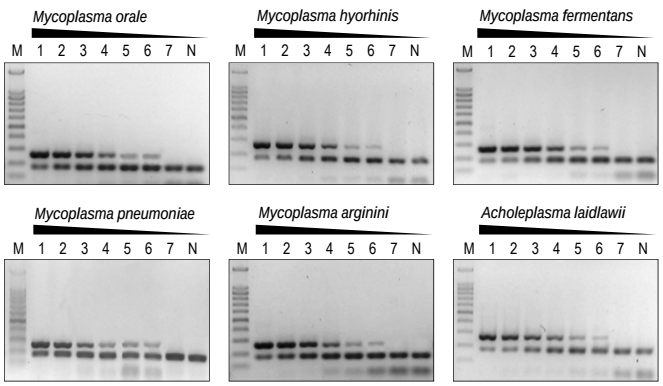


Fig. 2. Analytical Sensitivity of e-Myco™ VALiD Mycoplasma PCR Detection Kit
LaneM, SiZer™-100 DNA Marker**; Lane 1,** 1×10^6 cfu/ml of gDNA**; Lane 2,** 1×10^5 cfu/ml of gDNA**; Lane 3,** 1×10^4 cfu/ml of gDNA**; Lane 4,** 1×10^3 cfu/ml of gDNA**; Lane 5,** 1×10^2 cfu/ml of gDNA**; Lane 6,** 10 cfu/ml of gDNA**; Lane 7**, 1 cfu/ml of gDNA**; Lane N,** No template control.

◆ Specificity test

e-Myco™ VALiD Mycoplasma PCR Detection Kit is design to specifically detect only Mycoplasma spp. and provides high specificity resulted from no cross reactivity with other similar microorganisms. Figure 3 shows the evaluation data of e-Myco™ VALiD Mycoplasma PCR Detection Kit, suggesting that both internal control (app. 180 bp) and target bands (app. 270 bp) were detected. However, in cases of negative control(20 ng of non-mycoplasma bacterial genomic DNA, lanes 2~7) and no template control (lane N), only negative control band was detected.

[Sample information]

Lane	Name	ATCC No.
1	*Mycoplasma hyorhinis*	17981D-5
2	*Clostridium perfringens*	13124D-5
3	*Streptococcus mutans*	700610D-5
4	*Lactobacillus plantarum*	BAA-793D-5
5	*Mobiluncus mulieris*	35240D-5
6	*Gardnerella vaginalis*	49145D-5
7	*Haemophilus ducreyi*	700724D-5
8	*DNase/RNase Free Water*	-

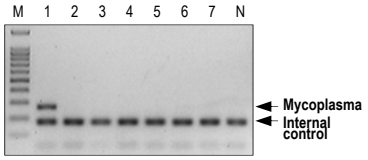


Fig. 3. Specificity of e-Myco™ VALiD Mycoplasma PCR Detection Kit
LaneM, SiZer™-100 DNA Marker**; Lane 1,** *Mycoplasma hyorhinis*; **Lane 2,** *Clostridium perfringens*; **Lane 3,** *Streptococcus mutans*; **Lane 4,** *Lactobacillus plantarum*; **Lane 5,** *Mobiluncus mulieris*; **Lane 6,** *Gardnerella vaginalis* **Lane 7**,*Haemophilus ducreyi*; **Lane N,** No template control.

◆ Comparison with direct plating method

e-Myco™ Mycoplasma PCR Detection Kit shows much higher sensitivity than conventional culture plate method, based on the direct comparison of PCR result done by this kit with the conventional colony counts, using 10-folds diluted Mycoplasma culture supernatant.

[Direct plating : *A. laidlawii*]

Dilution rate	Colony No.	Cell conc. (cfu/ml)
10^{-7}	92 ± 2	0.9×10^9
10^{-8}	12 ± 3.5	1.2×10^9
10^{-9}	1.3 ± 0.5	1.3×10^9

[PCR Detection : *A. laidlawii*]

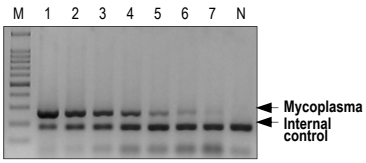


Fig. 4. Comparative test of e-Myco™ VALiD Mycoplasma PCR Detection Kit with direct plating method.
LaneM, SiZer™-100 DNA Marker**; Lane 1,** 10^{-3} diluted *A. laidlawii*; **Lane 2,** 10^{-4} diluted *A. laidlawii*; **Lane 3,** 10^{-5} diluted *A. laidlawii*; **Lane 4,** 10^{-6} diluted *A. laidlawii*; **Lane 5,** 10^{-7} diluted *A. laidlawii*; **Lane 6,** 10^{-8} diluted *of A. laidlawii*; **Lane 7**, 10^{-9} diluted *A. laidlawii*; **Lane N,** No template control.

◆ Elimination of Carryover Contamination

e-Myco™ VALiD Mycoplasma PCR Detection Kit contains 8-MOP (8-Methoxypsoralen), which prevents unfolding tendency of DNA double stand and immobilizes it when exposed under UV-irradiation for 10-20 minutes, leading to that gene amplification does not occur even if cross contaminations exist (fig 5).

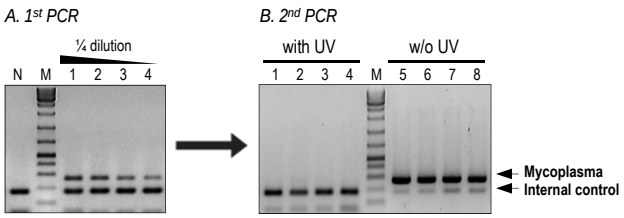


Fig. 5. Protection of cross-contamination using 8-MOP activation by UV irradiation of 1st PCR Product.
[Panel A] Lane M, SiZer™-1000 plus DNA Marker; **lane 1**, Internal control; **lane 2**, 50pg; **lane 3**, 10pg; **lane 4**, 2pg; **lane 5**, 0.4pg
[Panel B] lane M, SiZer™-1000 plus DNA Marker ; **lane 1, 5**, PCR product (1 µl) used from lane 1 of panel A; **lane 2, 6**, PCR product (1 µl) used from lane 2 of panel A; **lane 3, 7** , PCR product (1 µl) used from lane 3 of panel A; **lane 4, 8**, PCR product (1 µl) used from lane 4 of panel A.

ORDERING INFORMATION

Product Name	Amount	Cat. No.
e-Myco™ VALiD Mycoplasma PCR Detection Kit	48 Tubes	25239
M-Solution™ 1-2 Antibiotic for Mycoplasma	10 ml (each)	21081
SiZer™-100 DNA Marker	0.5 ml	24073
SiZer™-1000 plus DNA Marker	0.5 ml	24075

◆ References

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3. Pettersson et al., Sequence Analysis of 16S rRNA from Mycoplasmas by Direct Solid-Phase DNA Sequencing, Appl. Environ. Microbiol., 1994, 60 (7), 2456-2461.
4. European Pharmacopoeia. 6.1, section 2.6.7 Mycoplasma; Revised January 2008.
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