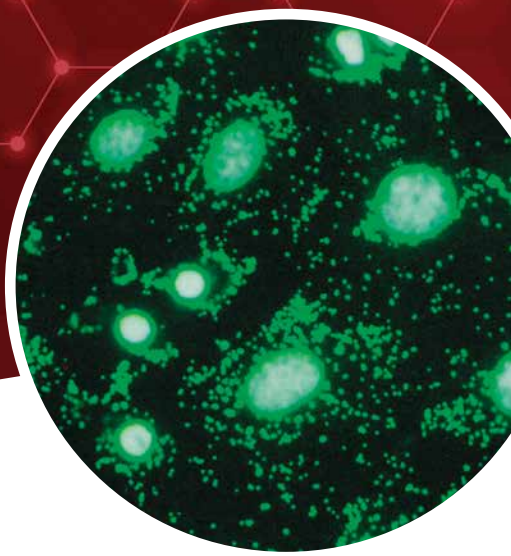


Myco-Sniff-Valid™
Mycoplasma PCR Detection Kit

Validated for sensitive, specific and rapid detection of mycoplasma

Cat. No. **093050301**

Size: **48 tests**

Storage: **-20 °C**

Revision Date: **2022-01**

INTRODUCTION

Mycoplasma, a genus of bacteria which lack a cell wall, are commonly found in research laboratories as contaminants in cell culture. This contamination can come from individuals in the lab or cell culture media ingredients. Mycoplasma contamination is a serious concern as it affects various cellular behaviors including metabolism, growth, viability, and morphology, and thus compromises the validity of experimental results and study data. Up to 30~85% of cell cultures may be contaminated by mycoplasma with the main species comprised of *M. orale*, *A. laidlawii*, *M. arginini* and *M. hyorhinis*. Therefore, testing for mycoplasma is an essential quality control step to assure accurate and reproducible results. Traditionally, a direct culture based method is used to detect for the presence of contaminating mycoplasma. However, this method is time-consuming (~28 days), inconsistent, and difficult to interpret. Recently, PCR-based detection methods have become an accepted standard protocol for mycoplasma detection to replace direct culture methods, including European Pharmacopoeia 2.6.7 and USP 63.

Myco-Sniff-Valid™ Mycoplasma PCR Detection Kit has been demonstrated to be a highly sensitive, specific and rapid method for the detection of mycoplasma contamination in cell cultures. Myco-Sniff-Valid™ Mycoplasma PCR Detection Kit is composed of a set of primers, which are specific for the highly conserved mycoplasma 16S-rRNA coding region, including *M. pneumoniae*, *M. arginini*, *M. hyorhinis*, *M. fermentans*, *M. orale* and *A. laidlawii*. This kit is specifically designed to detect the presence of mycoplasma that might contaminate biological materials such as cultured cells. The detection can be performed within 3 hours with a sensitivity as low as 10 CFU/mL.

An exogenous internal control is provided with the Myco-Sniff-Valid Mycoplasma PCR Detection Kit to distinguish negative reactions resulting from the absence of mycoplasma contamination from PCR inhibition. The primer sets included in the kit are used to amplify the internal control and target DNA, which are differentiated by size. Furthermore, a positive control sample is provided with this kit to verify the effectiveness of template DNA and confirm the size of PCR products for positive samples. 8-Methoxypsoralen (8-MOP) is also included in this kit to prevent cross-contamination by PCR products from previous experiments.

Each kit contains 48 PCR lyophilized tubes for 20 µL reactions.

KEY BENEFITS

- **Highly sensitive:** Detection limit as low as 10 CFU/mL.
- **Reliable:** Meets European Pharmacopoeia Guidance and Regulation. This test is suitable for product release testing and in-process control. It can replace culture and indicator cell tests.
- **Fast:** Replaces traditional 28-day culture testing with a 3 hour PCR test.
- **Wide detection range:** Detects common cell culture-infecting species of mycoplasma and also other various species of mycoplasma *(see Technical Guide)*.
- **Premixed for ease-of-use:** All PCR reaction components are included; just add template DNA or samples.
- **Highly specific:** No interference of animal or bacterial DNA.
- **Elimination of cross-contamination:** 8-MOP prevents cross-contamination from previous PCR products.
- **Exogenous internal control:** Helps differentiate false negatives due to PCR inhibition or erroneous PCR tests.
- **Sample control:** Easily verify the effectiveness of template gDNA by checking the amplification of the sample control DNA.

KIT STORAGE INFORMATION

Store at -20 °C. The kit has a stable shelf life of 1 year without showing any reduction in performance. The expiration date is labeled on the product box or Certificate of Analysis.

INTENDED USE

For Research Use Only, not for use in diagnostic procedures.

In-process monitoring for the presence of Mycoplasma. Prior to using it for other purposes, the user must validate the system in compliance with applicable laws, directives, and regulations.

KIT CONTENTS/DESCRIPTION

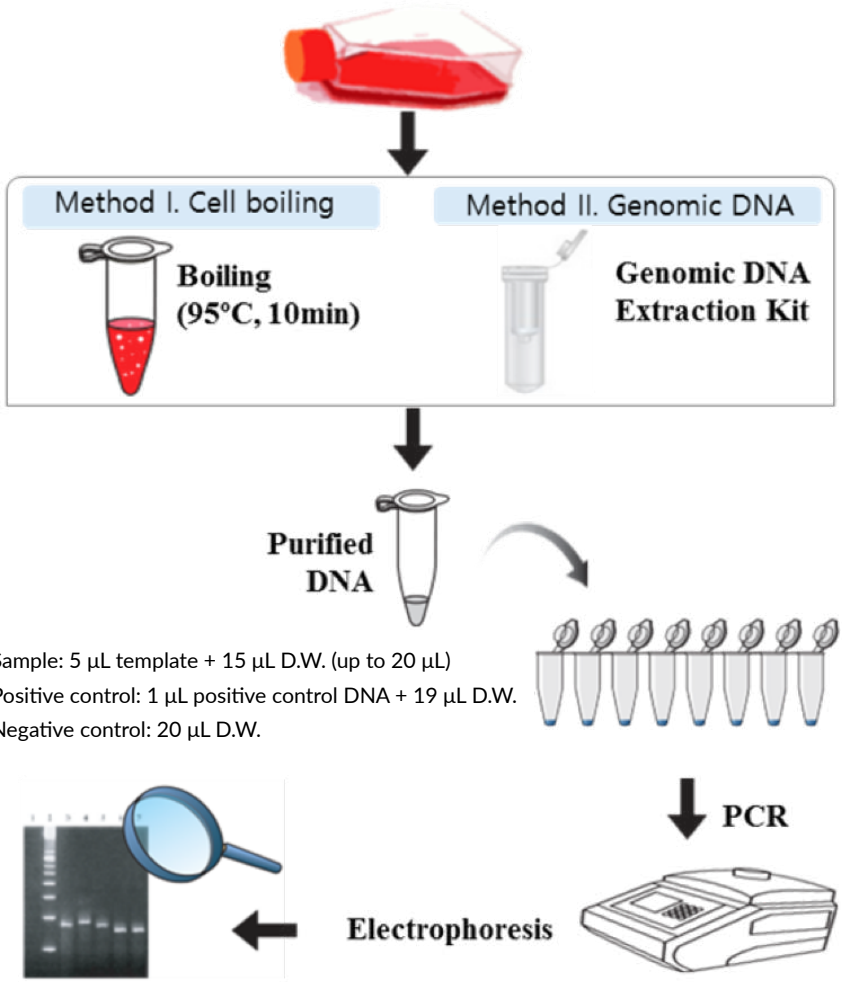
No	Contents	Composition	Qty
1	Myco-Sniff-Valid™ Mycoplasma PCR Premix	0.01% Hot Start Taq DNA Polymerase 0.01% dATP, dTTP, dGTP, dCTP 0.005% Mycoplasma Primers, Internal Control 0.001% 8-MOP (dissolved in DMSO)	48T
2	Control DNA	0.01% genomic DNA extracted from cultured human cells contaminated with *M. hyorhinis*.	25 µL x 3T
3	DNase/ RNase Free Water (D.W.)	No template control: DNase/RNase Free Water	1 mL x 1T

- **Myco-Sniff-Valid™ Mycoplasma PCR Premix:** Blue colored pellets in PCR Strips (48 tests)
- **Control DNA:** Colorless and transparent liquid
- **DNase/RNase Free Water:** Colorless and transparent liquid

ADDITIONAL REQUIRED MATERIALS *(NOT INCLUDED)*

- **Agarose**
- **Disposable gloves**
- **Pipettes and pipette tips (aerosol barrier)**
- **Electrophoresis equipment**
- **Thermal cycler**
- **Vortex mixer**

OVERVIEW OF MYCOPLASMA DETECTION



- Sample: 5 µL template + 15 µL D.W. (up to 20 µL)
- Positive control: 1 µL positive control DNA + 19 µL D.W.
- Negative control: 20 µL D.W.

SAMPLE PREPARATION

Method I: Cell Boiling Method *(the most commonly used)*

1. Prepare cell suspensions from test cell culture in a 1.5 mL tube. Count cell numbers using standard counting methods. A minimum of $5x10^4$ cells per test are required.

   NOTE ▸ *Strong mycoplasma infections are detected in as little as 10~100 cells, while weak infections require over 5,000~50,000 cells. Dilute the template according to the suspected infection rates. We recommend performing the PCR reaction after preparing serial dilutions of the supernatant to obtain optimal results.*

2. Transfer 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. We recommend repeating the wash step once more.

5. Resuspend the cell pellets in 100 µL of sterile PBS or DPBS solution.

   NOTE ▸ *For optimal results, use of PBS solution is recommended over Tris (10 mM, pH 8.5), TE (10 mM Tris, 0.1 mM EDTA), or autoclaved DW.*

6. Heat the samples at 95 °C for 10 min, then vortex for 5-10 sec. 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 reaction.

SAMPLE PREPARATION

Method II: Genomic DNA Extraction

- PCR inhibiting substances may accumulate over time in cell culture medium.
- Medium with more than 10-12% serum has inhibitory effects on downstream applications such as PCR. Moreover, phenol red, a routine material in cell culture medium, is likely to cross-react and thus interfere with the PCR signal detection.
- These negative effects can be overcome by using a general genomic DNA extraction kit for sample preparation or FastDNA™ SPIN Kit from MP Bio (SKU 116540600).
- Follow the protocol for genomic DNA extraction kit or FastDNA™ SPIN Kit.

Precautions before PCR Testing

- Leave the kit at 4 °C 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 performed on a clean bench that should be cleaned with 70% alcohol or 10% household bleach (Na-hypochlorite) after use. Samples should be prepared in a separate area from PCR reaction setup and use dedicated equipment. Samples are considered to be biological hazards and must be high-pressure sterilized prior to discarding.

PCR TEST PROTOCOLS

1. Prepare the appropriate number of Myco-Sniff-Valid Mycoplasma PCR Premix tubes. An appropriate number of tubes includes your sample, a positive control and a negative control.
2. Add 15 µL of DNase/RNase-free water into the PCR Premix tube.
3. Add 5 µL of DNA sample to each of the 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 allowing the mixture to stand at room temperature for 1-2 minutes after adding water.
6. Use the thermal cycling conditions presented in the table below for processing PCR reactions in a thermal cycler.

PCR Condition		Temp	Time
Initial denaturation		94 °C	1 min
X 40 Cycles	Denaturation	94 °C	30 sec
	Annealing	60 °C	20 sec
	Extension	72 °C	1 min
Final extension		72 °C	5 min

7. For analysis by electrophoresis, use 5 µL from each completed PCR reaction tube.
8. PCR products should be discarded after UV irradiation (10 min at 365 nm) to prevent carry-over contamination.

TECHNICAL INFORMATION

Interpretation

Myco-Sniff-Valid Mycoplasma PCR Detection Kit detects various Mycoplasma species with high sensitivity and specificity. For the validation of each PCR reaction, an internal control is included in each PCR Premix tube. The interpretation of experimental results is as follows:

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

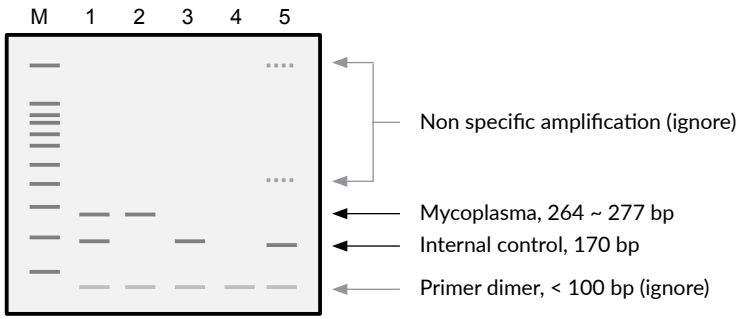


Figure 1. Example Data of Myco-Sniff-Valid Mycoplasma PCR Detection Kit

Lane	Mycoplasma	Test Results
1	Postive	**Valid**
2	Positive	**Valid** (Significant amount of target DNA)
3	Negative	**Valid** (Below 10 CFU/mL)
4	Unknown	**Invalid**, Retest is needed
5	Negative	**Valid** (low quality of template DNA or template degradation; ignore the non specific bands or primer dimer band)

Specificity Test

Myco-Sniff-Valid Mycoplasma PCR Detection Kit is designed to specifically detect only *Mycoplasma spp.* and provides high specificity due to a lack of cross reactivity with other similar microorganisms. Figure 2 shows the evaluation data of Myco-Sniff-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 samples (lane N), only internal control band was detected.

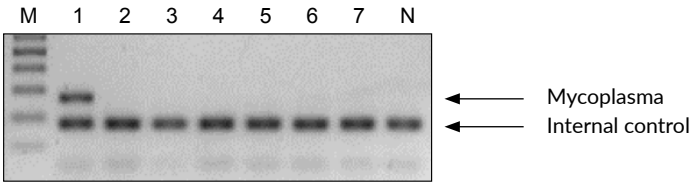


Figure 2. Specificity of Myco-Sniff-Valid Mycoplasma PCR Detection Kit

Lane M: 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.

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
N	DNase/RNase Free Water	

Analytical Sensitivity

Myco-Sniff-Valid Mycoplasma PCR Detection Kit is a highly sensitive and optimal solution for the efficient detection of *Mycoplasma spp.* contamination in cultures. To identify the analytical sensitivity, the genomic DNA from 6 *Mycoplasma spp.* cell cultures was purified. The sensitivity according to the DNA copy number was investigated after purifying gDNA from each cultured *Mycoplasma spp.*

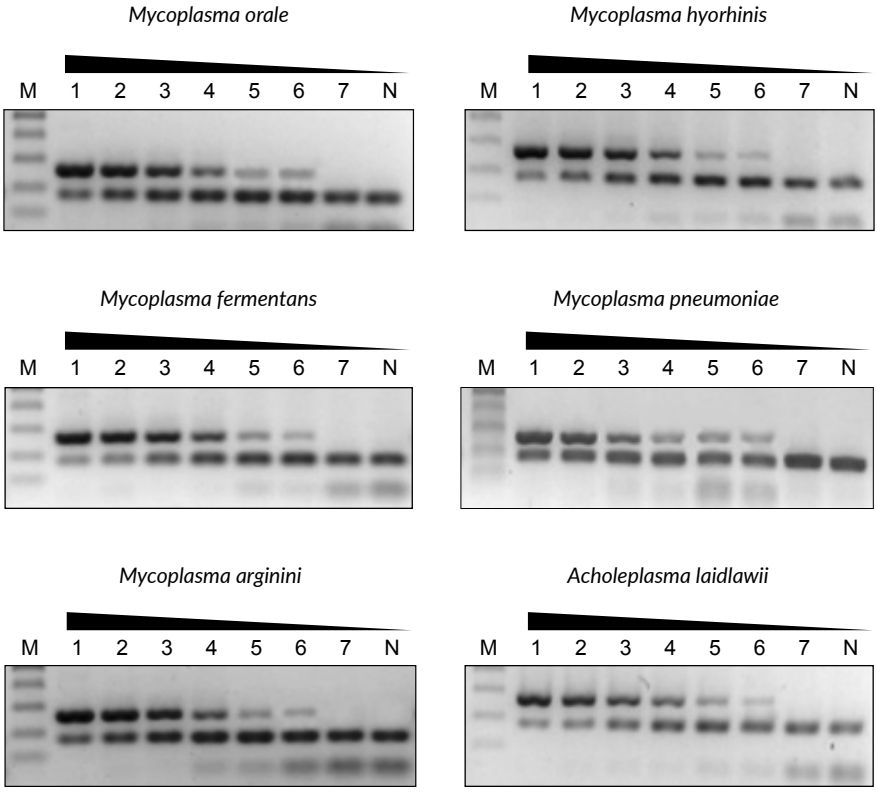


Figure 3. Analytical Sensitivity of Myco-Sniff-Valid Mycoplasma PCR Detection Kit

Lane M: 100 bp 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.

Comparison with direct plating method

Myco-Sniff-Valid Mycoplasma PCR Detection Kit shows much higher sensitivity than conventional culture plate methods. This is based on the direct comparison of PCR results performed with this kit to conventional colony counts using 10-fold dilutions of 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*]

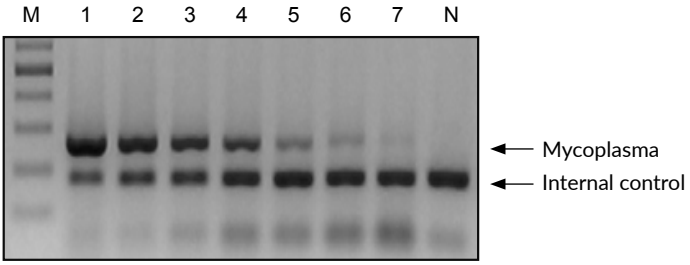


Figure 4. Comparative test of Myco-Sniff-Valid Mycoplasma PCR Detection Kit with direct plating method.

Lane M: 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.

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 pg. 11 of our Myco-Sniff Mycoplasma PCR Detection Kit User Manual. For more detailed species analysis, 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 sequence: AGGAT TAG ATA CCC TGG TAG TC-3' (22 mer)
- The PCR primers used in this kit differ from the sequencing primers. We do not list the PCR primer sequences contained in this kit.

TROUBLESHOOTING GUIDE

No Target band in positive reaction

Possible Causes	Comments & Suggestions
Check internal control band	If internal control band is present, then the issue is not with the PCR reaction.
Check the quality or concentration of template	If the PCR reaction is inhibited by impurities included in the DNA preparation, the use of diluted DNA template may be helpful. If the sample control (app. 570 bp length) and internal control (app. 160 bp length) are present and the target band is not visible, this indicates that the sample is not infected with mycoplasma.
Check PCR machine	The problem may be caused by the PCR machine. Check the temperature and ensure the machine was programmed correctly.

TROUBLESHOOTING GUIDE *(CONT.)*

No internal control band

Possible Causes	Comments & Suggestions
Verify template concentration	Competition can occur by using highly concentrated DNA template. Please repeat the PCR with a diluted template. If the concentration of template is above 50 ng, the signal of the internal control may be masked due to competition with the template. However, the signal of the sample control can still function to serve as an internal control.
Check the quality of template (for the possibility of contamination with PCR inhibitors)	If the PCR reaction is inhibited by impurities included in the DNA preparation, the use of diluted DNA as a template may be helpful. If the internal control band is absent, please inquire with our technical support staff.
Check the storage conditions of the product	Maintain appropriate preservation conditions.

Presence of amplified product in the negative control

Possible Causes	Comments & Suggestions
Check contamination of D.W.	D.W. may have become contaminated. Repeat PCR with fresh sterile water.
Check contamination of lab instruments and environments	We recommend using filter tips to reduce contamination and to sterilize pipettes prior to use. All procedures should be performed in dedicated environments free of contaminants.

Poor resolution on agarose gel

Possible Causes	Comments & Suggestions
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.

RELATED PRODUCT INFORMATION

Product Name	Size	Cat. No.
Mycoplasma Removal Agent (MRA)	5 mL	093050044
Myco-Sniff™ Mycoplasma PCR Detection Kit	48 tests	093050201
Myco-Sniff-Rapid™ Mycoplasma Luciferase Detection Kit	25 tests	093050401
	50 tests	093050402
Hoechst Mycoplasma Stain Kit	100 tests	093030000

NOTES

MP BIOMEDICALS

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