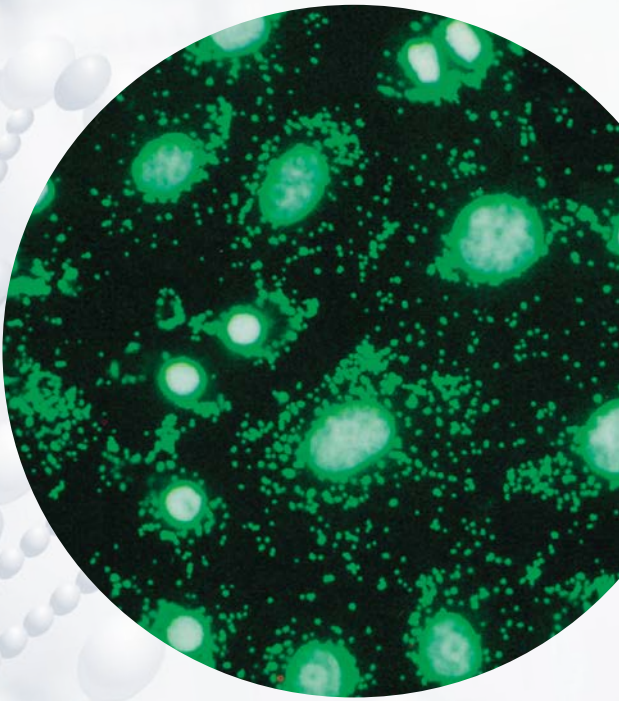
Instruction Manual

Myco-Sniff™

Mycoplasma PCR Detection Kit

Designed for sensitive, specific and rapid detection of mycoplasma



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RUO	Research Use only
REF	093050201

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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. Therefore, testing for mycoplasma is an essential quality control step to assure accurate and reproducible research. Although agar cultures and DNA fluorochrome staining methods can be used for mycoplasma detection, polymerase chain reaction (PCR) is the established method of choice for high-sensitivity detection.

Myco-Sniff™ 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. This kit greatly simplifies testing and detection of mycoplasma contamination in cell cultures with results in less than 3 hours. After PCR, the presence of contaminating mycoplasma can be easily detected by simply verifying the bands of amplified DNA fragments after gel electrophoresis.

Myco-Sniff Mycoplasma PCR Detection Kit can be used to detect a broader range of mycoplasma species compared to other commercially available PCR-based mycoplasma detection kits. The unique and specific primer sets used in this kit were designed from DNA sequences that code for highly conserved 16S rRNA from relevant mycoplasma species, thus preventing amplification of DNA from other sources. The high sensitivity of the kit allows for the detection of as little as 20 CFU/mL.

An exogenous internal control is provided with the Myco-Sniff 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 20 CFU/mL.
- **Wide detection range of mycoplasma:** Detect mycoplasma from over 8 genera, including 209 individual species.
- **Premixed for ease-of-use:** All PCR reaction components included; just add template DNA or samples.
- **Highly specific:** No interference of animal or bacterial DNA.
- **Fast:** Detection can be achieved within 3 hours.
- **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 a minimum 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

Kit Contents/Description

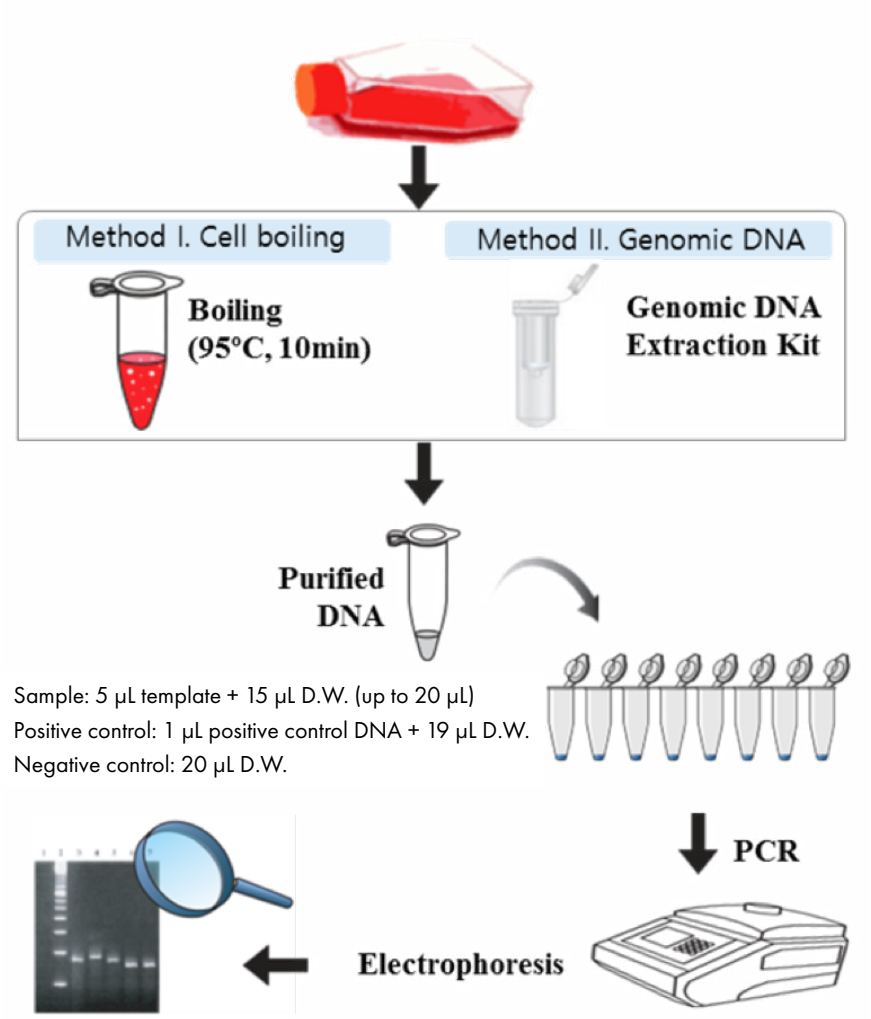
No	Contents	Composition	Quantity
1	Myco-Sniff™ Mycoplasma PCR Premix	0.01% Hot start Taq DNA Polymerase 0.01% dATP, dTTP, dGTP, dCTP 0.005% Mycoplasma Primers, Internal Control 0.001% 8-MOP (dissolved in DMSO)	48T
2	Control DNA	0.01% genomic DNA extracted from cultured human cells contaminated with *M. 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™ 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
- Electrophoresis equipment
- Pipettes and pipette tips (aerosol barrier)
- 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™ 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 35 Cycles	Denaturation	94 °C	30 sec
	Annealing	58 °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

- **Sample control:** indicates that the sample preparation and PCR reactions worked as expected
- **Target band:** indicates mycoplasma infection
- **Internal control:** indicates the absence of PCR inhibition or erroneous PCR results

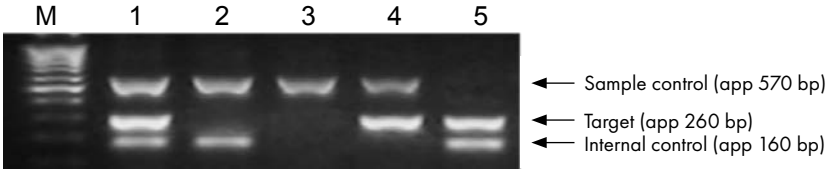


Fig. 1. Example Data of Myco-Sniff™ Mycoplasma PCR Detection Kit

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

Minimal amount of genomic DNA detectable

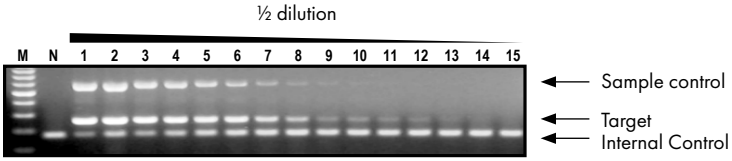


Fig. 2. Result of determining minimal required amount of genomic DNA per test
To determine the minimal required amount of genomic DNA, genomic DNA was isolated from a pure culture of *M. fermentans*-infected K562 cells using genomic DNA extraction kit. The isolated genomic DNA was serially diluted for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

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

Minimal cell number required

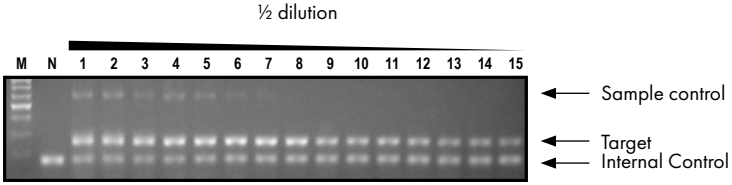


Fig. 3. Result of determining minimal required cell number per test
To determine the minimal required cell number, *M. fermentans*-infected K562 cells were grown in pure culture, serially diluted and tested. The result indicates that the detection limit with this kit is 15 cells per test.

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

DETECTABLE MYCOPLASMA STRAINS *(8 Genus/209 Species)*

Genus	Species	
Acholeplasma (5)	*Acholeplasma granularum* *Acholeplasma laidlawii* *Acholeplasma modicum*	*Acholeplasma morum* *Acholeplasma oculi*
Anaeroplasma (3)	*Anaeroplasma abactoclasticum* *Anaeroplasma bactoclasticum*	*Anaeroplasma varium*
Asteroleplasma (1)	*Asteroleplasma anaerobium*	
Entomoplasma (5)	*Entomoplasma lucivorax* *Entomoplasma luminosum* *Entomoplasma melaleucae*	*Entomoplasma somnilux* *Entoplasma ellychniae*
Mycoplasma (182)	*M. adleri* *M. agalactiae* *M. agalactiae (strain PG2)* *M. agassizii* *M. alkalescens* *M. alligatoris* *M. alvi* *M. amphoriforme* *M. anatis* *M. anseris* *M. arginini* *M. arthritidis* *M. auris* *M. bovigenitalium* *M. bovirhinis* *M. bovis* *M. bovoculi* *M. buccale*	*M. buteonis* *M. californicum* *M. canadense* *M. canimucosale* *M. canis* *M. capricolum* *M. capricolum subsp capricolum* *M. hyopharyngis* *M. hyopneumoniae* *M. caviae* *M. cavipharyngis* *M. citelli* *M. cloacale* *M. coccoides* *M. collis* *M. columbinasale* *M. columbinum*

Genus	Species	
Mycoplasma (182)	*M. columborale*	*Mycoplasma sp. Ms01*
	M. conjunctivae	*Mycoplasma sp. Ms02*
	M. corogypsi	*Mycoplasma sp. Ms03*
	M. cottewii	*M. hyopneumoniae (strain 232)*
	M. cricetuli	*Mycoplasma sp. PG50*
	M. crocodyli	*M. hyopneumoniae*
	M. cynos	*(strain 7448)*
	M. dispar	*M. insons*
	M. edwardii	*M. hyorhinis*
	M. elephantis	*M. lagogenitalium*
	M. equigenitalium	*M. imitans*
	M. equirhinis	*M. hyosynoviae*
	M. erythrodidelphis	*M. indiense*
	M. falconis	*M. iguanae*
	M. fastidiosum	*M. iowae*
	M. faucium	*M. iners*
	M. felifaucium	*M. leocaptivus*
	M. feliminutum	*M. leonicaptivi*
	M. felis	*M. leopharyngis*
	M. fermentans	*M. lipofaciens*
	M. flocculare	*M. lipophilum*
	M. gallinaceum	*M. microti*
	M. gallinarum	*M. moatsii*
	M. gallisepticum	*M. mobile*
	M. gallopavonis	*M. molare*
	M. gateae	*M. monodon*
	M. genitalium	*M. muris*
	M. genitalium G37	*M. mustelae*
	M. glycophilum	*M. mycoides*
	M. gypis	*M. mycoides subsp. capri*
	M. haemocanis	*M. mycoides subsp.*
	M. haemofelis	*mycoides LC*
	M. haemolama	*M. mycoides subsp.*
	M. haemomuris	*mycoides SC*
	M. hominis	*M. mycoides sunsp. capri*

Genus	Species	
Mycoplasma (182)	*M. neurolyticum*	*M. zalophi*
	M. opalescens	*M. zalophidermidis*
	M. orale	*Mycoplasma sp. Saa1e*
	M. ovipneumoniae	*Mycoplasma sp. Z61*
	M. oxoniensis	*Mycoplasma sp. SF12*
	M. penetrans	*Mycoplasma sp. 07SH-h*
	M. phocicerebrale	*Mycoplasma sp. 07SH-p*
	M. phocidae	*Mycoplasma sp. 10T3*
	M. phocirhinis	*Mycoplasma sp. 10T4*
	M. pirum	*Mycoplasma sp. 11CL2*
	M. pneumoniae	*Mycoplasma sp. 1220*
	M. primatum	*Mycoplasma sp. 13CL*
	M. pullorum	*Mycoplasma sp. 15CL2*
	M. pulmonis	*Mycoplasma sp. 237IAT*
	M. putrefaciens	*Mycoplasma sp. 2F1AT*
	M. salivarium	*Mycoplasma sp. 34CL*
	M. simbae	*Mycoplasma sp.39CL*
	M. spermatophilum	*Mycoplasma sp. 50587*
	M. maculosum	*Mycoplasma sp. 8790CV*
	M. meleagridis	*Mycoplasma sp. 94630*
	M. sphenisci	*Mycoplasma sp. A1802T*
	M. spumans	*Mycoplasma sp. ARNO*
	M. sturni	*Mycoplasma sp. 'bovine*
	M. sualvi	*group 7'*
	M. subdolum	*Mycoplasma sp. C3T*
	M. suis	*Mycoplasma sp. China-1*
	M. synoviae	*Mycoplasma sp. CSL 4779*
	M. synoviae (strain 53)	*Mycoplasma sp.*
	M. testudineum	*CSL 7518-lung*
	M. testudinis	*Mycoplasma sp. VJC358*
	M. timone	*Mycoplasma sp. HRC689*
	M. verecundum	*Mycoplasma sp. IS2505*
	M. vulturii	*Mycoplasma sp. M1*
	M. wenyonii	*Mycoplasma sp. M200-2*
	M. yeatsii	*Mycoplasma sp. M209-7*

Genus	Species	
Mycoplasma (182)	*Mycoplasma sp. M209-8* *Mycoplasma sp. M221-9* *Mycoplasma sp. M222-2* *Mycoplasma sp. M222-5* *Mycoplasma sp. M26* *M. capricolum subsp. capripneumoni* *M. capricolum subsp. capripneumoniae*	*Mycoplasma sp. ovine/caprine serogroup 11* *M. hyopneumoniae (strain J / ATCC 25934)* *Mycoplasma. sp. 'feline hemotropic Switzerland'* *Mycoplasma sp. Saa1c* *Mycoplasma sp. SF9*
Mesoplasma (3)	*Mesoplasma entomophilum* *Mesoplasma florum*	*Mesoplasma lactucae*
Spiroplasma (9)	*Spiroplasma apis* *Spiroplasma citri* *Spiroplasma CN-5* *Spiroplasma DU-1* *Spiroplasma DW-1*	*Spiroplasma gladiatoris* *Spiroplasma mirum* *Spiroplasma MQ-1* *Spiroplasma taiwanense*
Ureaplasma (1)	*Ureaplasma urealyticum*	

Important Notes

- The sequence of amplified PCR products differs slightly from species to species. Sequencing analysis can be approximately determined for mycoplasma species using the primer indicated below. Please refer to the phylogenetic table on the previous page. For more detailed species analysis, perform additional PCR reactions with your designed primers.
- Sequencing primer sequences: GGA TTA GAT ACC CTG GTA GTC CAC G-3′ (25 mer). Note: We list only the Forward primer sequences. Please synthesize the primer, and then analyze by general sequencing methods.
- The PCR primers used in this kit (sequencing is not listed) differ from the sequencing primer.
- The PCR conditions were optimized to obtain the highest level of sensitivity of target gene detection. Therefore, the internal control band or sample control band may sometimes appear faint depending on the efficiency of target gene amplification. This efficiency is dependent upon the amount of template DNA added to the reaction. Please refer to the following table for additional information on this dependency.

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

Troubleshooting Guide

Issue	Possible Causes	Comments & Suggestions
No target band in positive reaction	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.

Issue	Possible Causes	Comments & Suggestions
No internal control band	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.

Issue	Possible Causes	Comments & Suggestions
Presence of amplified product in the negative control	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.
No sample control band	Check template concentration	Sample control band may become undetectable 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 primer sets included in this kit can amplify a human-specific DNA sequence. If the template source is not human cells, the amplification of sample control will not occur.

Related Product Information

Product Name	Amount	Cat. No.
Mycoplasma Removal Agent (MRA)	5 mL each	093050044
Myco-Sniff™ Mycoplasma PCR Detection Kit	48 tests	093050201
Myco-Sniff-Valid™ Mycoplasma PCR Detection Kit	48 tests	093050301

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Distributor pro Českou republiku a Slovensko:

M.G.P. spol. s r.o.
Kvítková 1575
Zlín 760 01
Česká republika
tel. +420 577 212 140
e-mail: mgp@mgp.cz

MGP spol. s r.o.
Šustekova 2
Bratislava 851 04
Slovensko
tel. +421 254 654 841
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