

## Product Information

### LookOut® Mycoplasma qPCR Detection Kit

Catalog Number **MP0040A**

Storage Temperature 2–8 °C

## TECHNICAL BULLETIN

### Product Description

The LookOut® Mycoplasma PCR Detection Kit utilizes the polymerase chain reaction (PCR), which is established as the method of choice for highest sensitivity in the detection of *Mycoplasma* and *Acholeplasma* contamination in cell cultures and other cell culture derived biologicals.

The primer/probe system detects the highly conserved 23S rRNA operon coding region of the mycoplasma genome. The kit is highly specific and does not detect eukaryotic DNA. The detection spectrum includes most mycoplasma species identified as cell culture contaminants (see Table 2). The kit contains the nucleotide dUTP instead of dTTP and is, therefore, suitable for UNG pretreatment.

The kit contains Internal Control DNA (an optional Inhibition Control). The Internal Control DNA can be added to the MasterMix to detect incomplete PCR or directly to a sample prior to complete DNA isolation and the purification process. An internal control probe is already included in the Mycoplasma Mix. This probe emits fluorescent light with amplification of the Internal Control DNA confirming mycoplasma-negative, but successfully performed test reactions.

This kit already contains *Taq* polymerase in the Mycoplasma Mix.

### Components

- Mycoplasma Mix 1 each  
Catalog Number MPD005  
lyophilized primers, probe, and deoxynucleotide triphosphates dATP, dCTP, dGTP, and dUTP, *Taq* polymerase, aliquoted for 25 reactions
- Rehydration Buffer (1.8 ml) 1 each  
Catalog Number MPD004

- Positive Control DNA 1 each  
Catalog Number MPD003  
non-infectious, lyophilized Plasmid DNA
- Internal Control DNA 1 each  
Catalog Number D5072  
non-infectious, lyophilized synthetic DNA
- PCR Grade Water 1 each  
Catalog Number W0522  
water for resolving the components and setting up the master mix

### Equipment and Reagents Required but Not Provided

- qPCR instrument
- Corresponding PCR reaction tubes
- Microcentrifuge, micropipettes, and filtered tips (1–1,000 µl)

### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

### Preparation Instructions

#### Reconstitution of the Reagents

1. Centrifuge tubes with lyophilized components (5 seconds at maximum speed)
2. Add 575 µl of Rehydration Buffer to the Mycoplasma Mix
3. Add 300 µl of PCR Grade Water to each Positive Control and Internal Control
4. Incubate for 10 minutes at room temperature
5. Vortex and centrifuge again

### Storage/Stability

Kit components are stable during shipping at ambient temperature. Upon receipt, store at 2–8 °C.

After reconstitution of the Mycoplasma Mix, the Positive Control, and the Internal Control, store at –20 °C and avoid repeated freezing and thawing. For repeated testing of low sample numbers, Mycoplasma Mix and controls should be aliquoted after reconstitution.

### Procedure

#### Preparation of Sample Material

Cell lines should be pre-cultured in the absence of mycoplasma active antibiotics for several days to maximize test sensitivity. Samples should be derived from cultures, which are at 90–100 % confluence. PCR inhibiting substances may accumulate in the medium of older cultures. For a sample from an older culture, a DNA extraction is strictly recommended prior to testing. The GenElute™ Blood Genomic DNA Kits (Catalog Numbers NA2010 and NA2020) are recommended.

Penicillin and streptomycin in the culture medium do not inhibit mycoplasma nor affect test sensitivity. Cell culture supernatant is preferred to test for mycoplasma. Cell pellets should only be tested after suitable DNA extraction, since debris will interfere with the PCR reaction. With average titers at  $10^6$  particles/ml and a maximum titer at  $10^8$  particles/ml, sufficient mycoplasma is found in the supernatant to guarantee a sensitive PCR. However, other materials that can be tested are fetal calf serum, vaccines, and paraffin-embedded samples following DNA extraction. If necessary, templates for PCR analysis are prepared by DNA extraction. Please be sure to remove any alcohol containing wash buffer from the preparation to avoid coelution of alcohol and sample material. Any remaining alcohol may inhibit the PCR. 2 µl of the extract can be used directly as PCR template.

To avoid false positive results, the use of the PCR grade water delivered with the kit, aerosol-preventive filter tips, and gloves is recommended.

Sample material may be prepared by one of the following methods:

#### A. Heat-inactivation of the sample material

The templates for the PCR analysis are prepared by direct heating of the cell culture supernatant or the biological sample material:

1. Transfer 100 µl of supernatant from the test culture to a sterile tube. The lid should be tightly sealed to prevent opening during heating.
2. Boil or incubate the sample supernatant at 95 °C for 10 minutes.
3. Briefly centrifuge (5 seconds at  $1,000 \times g$ ) the sample supernatant to pellet cellular debris before adding to the PCR mixture. The templates are stable at 2–8 °C for at least 1 week.

The supernatant is used in the PCR. Alternatively, the DNA can be purified with a commercial extraction kit.

#### B. Enrichment of mycoplasma by centrifugation

1. Transfer 1 ml of supernatant from the test culture to a sterile tube. The lid should be tightly sealed to prevent opening during heating.
2. Centrifuge the supernatant (15 minutes at  $10,000 \times g$ ) to sediment mycoplasma particles. Alternatively, centrifuge the supernatant 6 minutes at  $13,000 \times g$ .
3. Remove the supernatant and suspend the pellet in 50 µl of buffer (10 mM Tris, pH 8.4).
4. The sample should be vortexed and finally heated up to 95 °C for 10 minutes.

The extracts can be stored at –20 °C for a period of one year. Repeated freezing and defrosting, or storage in the refrigerator for longer than 12 months should be avoided. The sample should not contain more than 100 µg/ml DNA.

### The PCR Master Mix

Total volume per reaction is 25 µl. When setting up reactions, calculations should also include positive and negative controls.

	<b>1 reaction</b>	<b>25 reactions</b>
Mycoplasma Mix	22 µl	550 µl
Internal Control DNA	1.0 µl	25 µl
+ template DNA, Negative control or Positive control	2.0 µl	

Pipette 23 µl of Master Mix into a 1.5 ml PCR reaction tube and mix gently.

The total duration from Master Mix preparation to PCR cycling must not exceed 60 minutes to avoid a decrease in the fluorescent signal.

Add 2 µl of prepared sample to PCR reaction tube per sample being tested. After pipetting the negative control (2 µl of water or negative control of DNA extraction/reaction), the tube must be sealed before proceeding with the samples. Also pipetting of the samples and sealing the tubes must be completed before proceeding with the positive control (2 µl/reaction) in order to avoid cross contamination.

### Programming of the Cycler

Program Step 1: Pre-incubation

Setting: Hold

Temperature: 95 °C

Incubation time: 3:00 min

Program Step 2: Amplification

Cycles 45

Denaturing 96 °C for 30 seconds

Annealing & Reading 55 °C for 30 seconds

Extension 60 °C for 30 seconds

For all vials set detectors to FAM for Mycoplasma Target Probe and to HEX for the Internal Control Probe. No quencher must be specified. Inactivate available ROX Reference functions. Fluorescence should be measured during extension.

### **Results**

A successfully performed PCR without inhibition is indicated by an increasing fluorescence signal in the internal control channel (HEX), provided the Internal Control was added to the Master Mix. The Internal Control can be detected with an orange filter (535-555 nm for HEX). The presence of mycoplasma DNA in the sample is indicated by an increasing fluorescence signal at 520 nm (FAM) and is usually detected with a green filter (470–510 nm).

Mycoplasma DNA and Internal Control DNA are competitors in PCR. Because of the very low concentration of Internal Control in the PCR mix, the signal strength in this channel is reduced with the increasing mycoplasma DNA loads in the sample. False-negative results, e.g., due to inhibition of the reaction by the sample matrix, can be detected individually for each sample as these reactions do not show any fluorescence signal.

**Table 1.**

Interpretation of PCR Results

<b>Mycoplasma PCR</b>	<b>Internal Control</b>	<b>Interpretation</b>
Positive	Irrelevant	Mycoplasma positive
Negative	Negative	PCR inhibition
Negative	Positive	Mycoplasma negative

### Troubleshooting

- No amplification of the control DNA may be due to the following:
  - programming error
  - degradation of the control DNA due to improper storage or contamination with nucleases
  - pipetting error
- Before rerunning of a negative and a positive control, check thermocycler program and pipetting scheme.
- If the PCR of a sample is inhibited, PCR inhibitors can easily be removed from the sample by performing a DNA extraction with the GenElute Blood Genomic DNA Kit.

**Table 2.**

Species specificity includes the following:

<i>A. laidlawii</i>	<i>M. agalactiae</i>	<i>M. hyorhinis</i>	<i>M. hyosynoviae</i>
<i>M. agassizii</i>	<i>M. alkalescens</i>	<i>M. iguanae</i>	<i>M. indiense</i>
<i>M. anseris</i>	<i>M. arginini</i>	<i>M. iners</i>	<i>M. lagogenitalium</i>
<i>M. arthritidis</i>	<i>M. bovigenitalium</i>	<i>M. lipofaciens</i>	<i>M. maculosum</i>
<i>M. bovirhinis</i>	<i>M. bovis</i>	<i>M. melegridis</i>	<i>M. moatsii</i>
<i>M. buccale</i>	<i>M. buteonis</i>	<i>M. opalescens</i>	<i>M. orale</i>
<i>M. californicum</i>	<i>M. canadense</i>	<i>M. pneumoniae</i>	<i>M. pulmonis</i>
<i>M. capricolum</i>	<i>M. caviae</i>	<i>M. salivarium</i>	<i>M. simbae</i>
<i>M. citelli</i>	<i>M. cloacale</i>	<i>M. sp. ovine/caprine</i>	<i>M. spermatophilum</i>
<i>M. collies</i>	<i>M. columbinasale</i>	<i>M. sphenisci</i>	<i>M. spumans</i>
<i>M. columbinum</i>	<i>M. columborale</i>	<i>M. sualvi</i>	<i>M. subdolum</i>
<i>M. cricetuli</i>	<i>M. cynos</i>	<i>M. synoviae</i>	<i>M. testudineum</i>
<i>M. edwardii</i>	<i>M. equirhinis</i>	<i>M. timone</i>	<i>M. turnidae</i>
<i>M. falconis</i>	<i>M. faucium</i>	<i>M. verecundum</i>	<i>M. zalophi</i>
<i>M. felifaucium</i>	<i>M. fermentans</i>		
<i>M. gallinaceum</i>	<i>M. gallinarum</i>		
<i>M. gallopavonis</i>	<i>M. gateae</i>		
<i>M. glycophilum</i>	<i>M. gypis</i>		
<i>M. hominis</i>	<i>M. hyopharyngis</i>		

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BG,JF,MAM 03/17-1