3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

LookOut® Mycoplasma PCR Detection Kit

Catalog Number **MP0035**
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. Detection requires ≥20 mycoplasma genomes per 2 µL of sample.

The primer set is specific to the highly conserved 16S rRNA coding region in the mycoplasma genome. This allows for detection of all mycoplasma species tested so far and usually encountered as contaminants in cell cultures (see Appendix). This kit is **not suitable** for detection of *M. pneumoniae, U. urealyticum,* or other clinically associated species. Eukaryotic and bacterial DNA are not amplified.

Because the reaction tubes included with the kit are pre-coated with appropriate dNTPs and primers, the total assay time is greatly reduced compared to general protocols that require individual loading of reaction tubes. The reaction tubes also contain DNA to serve as an internal control. A successfully performed reaction is indicated by a distinct 481 bp band on the agarose gel for the internal control DNA. For convenience, the gel loading buffer and dye are already included in the reaction tubes. After thermal cycling the PCR can be loaded directly on an agarose gel.

This kit has been tested and optimized for use with JumpStart™ *Taq* DNA Polymerase, Catalog Number D9307. It is **highly recommended** that this product be used with the kit. Use of other *Taq* polymerase products may require reaction optimization to achieve proper results.

Components

- Test Reaction Tubes (Catalog Number T0701) – 3 strips of 8 tubes each (tubes pre-coated with primers, dNTPs, internal control DNA, and gel loading buffer/dye)
- Positive Control PCR Reaction Tubes (Catalog Number P9123) – 1 strip of 8 tubes (tubes pre-coated with primers, dNTPs, internal control DNA, non-infectious DNA fragments of *Mycoplasma orale* genome prepared by PCR, and gel loading buffer/dye)
- Rehydration Buffer (Catalog Number M4694) – 1.2 ml
- Caps for PCR Reaction Tubes (Catalog Number C0242) – 4 strips of 8 caps

Equipment and Reagents Required But Not Provided

- JumpStart *Taq* DNA Polymerase, Catalog Number D9307
- amplification tubes
- GenElute™ Blood Genomic DNA Kit, Catalog Number NA2010 (optional, for DNA extraction and purification)
- 1.2% Agarose Gel

Precautions and Disclaimer

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

Storage/Stability

Kit components are stable during shipping at ambient temperature. Upon receipt, store at 2–8 °C. The kit is stable until the expiration date stated on the label.

Procedure

A. 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 that 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 Kit is recommended.

To avoid false positive results, the use of deionized, DNA-free water, aerosol-preventive filter tips, and gloves is recommended.

Cell culture supernatants can be tested directly without prior preparation. Stable templates for PCR analysis at a later date can be prepared by boiling the supernatant of cell cultures or other biologicals for 5 minutes as follows:

1. Transfer 100 µL of supernatant from the test culture to a sterile amplification tube. The lid should be tightly sealed to prevent opening during heating.
2. Boil or incubate the sample supernatant at 95 °C for 5 minutes.
3. Briefly centrifuge (5 seconds) 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.

B. PCR Setup

Total volume for each PCR is 25 µL. It is recommended to perform a positive (step 4) and a negative control reaction.

1. DNA Polymerase/Rehydration Buffer Preparation – Determine the total volume of DNA Polymerase/ Rehydration Buffer required for the reactions (see Table 1). Calculations should also include an additional reaction volume (23 µL) to compensate for pipetting losses. Pipette the required volume of Rehydration Buffer into a clean amplification tube and add the required volume of DNA Polymerase. One unit of DNA Polymerase is required per reaction. For JumpStart *Taq* DNA Polymerase with 2.5 units/µL, a volume of 0.5 µL is required per reaction. Mix the DNA Polymerase/Rehydration Buffer carefully by flicking the tube. DO NOT VORTEX!

Table 1.

	Sample	Positive Control	Negative Control
DNA Polymerase/ Rehydration Buffer	23 µL	25 µL	23 µL
Sample Volume	2 µL	–	–
DNA-free Water	–	–	2 µL

2. Test Reaction Tube rehydration - Remove strip of Test Reaction Tubes (transparent) from bag and cut off the appropriate number of tubes for the Negative Control and Samples. Replace remaining tubes in bag and seal. Peel off protective film from tubes. Add 23 µL of the prepared DNA Polymerase/Rehydration Buffer to each Test Reaction Tube.
3. Negative Control/Sample addition - Add 2 µL of DNA-free water to the Negative Control and 2 µL of sample to each Sample Reaction Tube. Close tubes with Caps for PCR Reaction Tubes included in kit. Label tubes as appropriate.
4. Positive Control Preparation - Remove strip of Positive Control Reaction Tubes (pink) from bag and cut off the appropriate number of tubes. Replace remaining tubes in bag and seal. Peel off protective film from tubes. Label tubes as appropriate. Add 25 µL of the prepared DNA Polymerase/Rehydration Buffer to each tube. Close tubes with Caps for PCR Reaction Tubes included in kit.
5. Incubation - Mix contents of each tube thoroughly by flicking tubes. DO NOT VORTEX! Collect liquid contents at the bottom of the PCR tube. Incubate at room temperature for 5 minutes. Proceed immediately to thermal cycling.

C. Thermal Cycler Profile

The programming process of your cycler is explained in the instrument manual.

1. The incubation time depends on the DNA polymerase used. Hot start enzymes need to be activated at 94 °C. Please see DNA polymerase data sheet for duration. No activation step is required for JumpStart *Taq* DNA polymerase.

   Thermal Cycler Program
   1 cycle 94 °C for 2 minutes

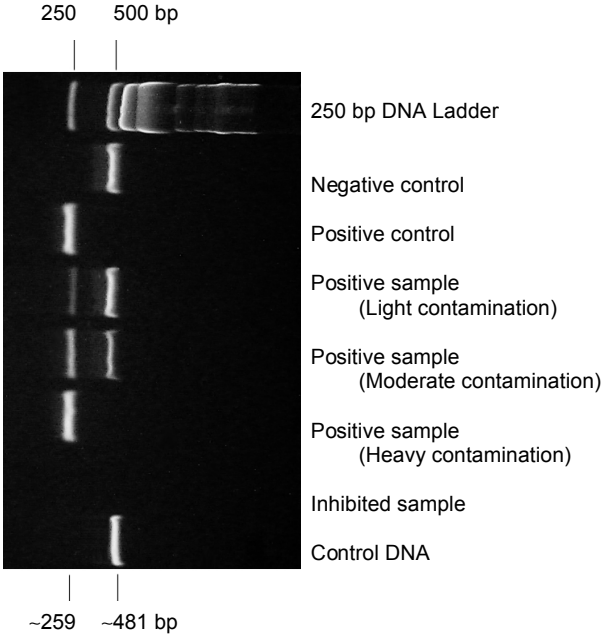
   40 cycles 94 °C for 30 seconds
   55 °C for 30 seconds
   72 °C for 40 seconds
   cool down to 4–8 °C

D. Agarose Gel
1. Use 1.2% standard agarose gel with 5 mm comb.
2. Directly load 8 µL for each PCR into a separate lane. The loading buffer is included in the PCR mixture.
3. Stop electrophoresis after migration of 2.5 cm (depending on the electrophoresis chamber used, e.g., run for 25 minutes at 100 V).

Results

Gel Evaluation (Figure 1)
1. The negative control samples show a distinct 481 bp band. Internal controls should appear in every lane indicating a successfully performed PCR. This band may be less intense with increased amounts of amplicons formed, caused by mycoplasma DNA loads of $>5 \times 10^4$ copies/ml.
2. The positive control shows a band at 259 bp and depending on the concentration of mycoplasma DNA, an additional band at 481 bp due to the internal control.
3. Mycoplasma positive samples show bands in the range of 260 ± 8 bp.

Figure 1.
Relevant Amplicon Bands



Troubleshooting
1. No amplification of the control DNA may be due to the following:
   - programming error
   - insufficient activity of DNA *Taq* polymerase
   - reaction buffer is not suitable for polymerase used (other than JumpStart *Taq* DNA Polymerase)
   - pipetting error
2. Before rerunning of a negative and a positive control, check thermocycler program and pipetting scheme. DNA polymerase concentration can be raised up to 2.5 units/reaction.
3. This kit has been designed for high sensitivity and is prone to nonspecific annealing. This may result in less intense bands of various lengths being produced that are not indicative of positive results. Possible primer self-annealing produces another band of 80–90 bp, but also does not affect the precision or results of the test.
4. 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.

Appendix

Species specificity includes the following:

A. laidlawii	*M. agalactiae*
M. arginini	*M. arthritidis*
M. bovis	*M. cloacale*
M. falconis	*M. faucium*
M. fermentans	*M. hominis*
M. hyorhinis	*M. hyosynoviae*
M. opalescens	*M. orale*
M. primatum	*M. pulmonis*
M. salivarium	*M. spermatophilum*
M. timone	

This kit is not suitable for detection of *M. pneumoniae, U. urealyticum,* or other clinically associated species.

LookOut is a registered trademark, and JumpStart and GenElute are trademarks of Sigma-Aldrich Co. LLC.

BG,DF,JF,GS,PHC,MAM 01/16-1