User Guide

LookOut® One-Step Mycoplasma PCR Detection Kit

MP0050-25TST (25 reactions)
MP0050-100TST (100 reactions)

Storage Temperature 2–8 °C

Product Description

The LookOut® Mycoplasma PCR Detection Kit utilizes the polymerase chain reaction (PCR), which was established as the method of choice for highest sensitivity in the detection of *Mycoplasma* and *Acholeplasma* contamination in cell cultures and other culture-derived biologicals. Detection requires as little as 10-20 fg of mycoplasma DNA corresponding to 10-20 mycoplasma per sample volume.

The primer set is specific to the highly conserved rRNA operon, or the 16S rRNA coding region in the mycoplasma genome. This allows for detection of all *Mycoplasma*, *Acholeplasma*, and *Ureaplasma* species tested so far, which are typically encountered as contaminants in cell cultures.

Eukaryotic and bacterial DNA are not amplified by this kit.

Just one protocol is needed for the detection of all mycoplasma species. The detection procedure can be performed within 3 hours. The PCR mix also provides internal control DNA, which results in a 190 bp band on the agarose gel to indicate a successfully performed reaction.

Precautions and Disclaimer

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

Components

Description	Quantity	Catalogue Number
PCR Mix, aliquoted for 25 reactions:	1 vial	MP0050-25TST
	4 vials	MP0050-100TST
Lyophilized Taq polymerase, primers and deoxynucleotide triphosphates dATP, dCTP, dGTP and dUTP		
Reaction Buffer, 1.3 mL	1 vial	MP0050-25TST
	2 vials	MP0050-100TST
Positive Control DNA	1 vial	
Lyophilized synthetic DNA, non-infectious		
Water, 2 mL	1 vial	

Storage/Stability

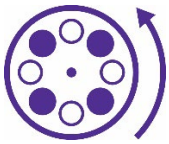
Kit components are stable during shipping at ambient temperature. Upon receipt, store at 2-8 °C. After rehydration of the PCR mix, the positive control, and the internal control, store below –18 °C and avoid repeated freezing and thawing. For repeated testing of a few samples at a time, PCR mix and controls should be aliquoted after rehydration. By following these recommendations, the kit is stable until the expiration date stated on the label.

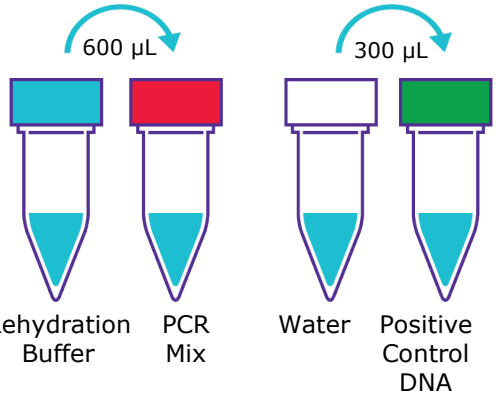
Quick Start Guide

This procedure overview is not a substitute for the detailed instructions.

Reagent Preparation

Centrifuge PCR Mix and Positive Control DNA





Incubate for 5 min at room temperature

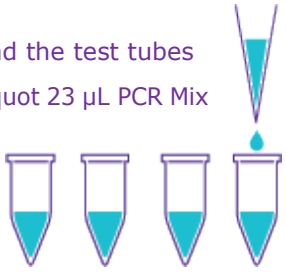
Vortex briefly

Centrifuge for 5 seconds

Preparation of PCR Reactions

Load the test tubes

Aliquot 23 µL PCR Mix

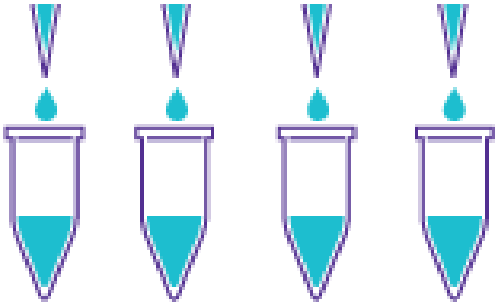


Add 2 µL Sample

Add 2 µL Sample

Add 2 µL Positive Control DNA

Add 2 µL Fresh Cell Culture Medium



Centrifuge briefly

Start PCR Reaction

1 cycle	94 °C for 2 min
39 cycles	94 °C for 30 sec
	55 °C for 30 sec
	72 °C for 30 sec
Hold at	4-8 °C

Procedure

Preparation of Sample Material

Cell lines should be pre-cultured in the absence of 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 these sample materials a DNA extraction is strictly recommended prior to testing.

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

Templates for PCR analysis are prepared by boiling the supernatant of cell cultures or other biologicals for 10 minutes as follows:

1. Transfer 100 µL of supernatant from the test culture to a sterile microcentrifuge tube (T0447). The lid should be tightly sealed to prevent opening during heating.
2. Heat the sample supernatant at 95 °C for 10 minutes.
3. Briefly centrifuge (5 seconds) the sample supernatant to pellet cellular debris before adding to the PCR mixture.

Rehydration of the Reagents

1. Before rehydrating the tubes, centrifuge the tubes to ensure that the lyophilized components are spun down (5 seconds at maximum speed).
2. Add the appropriate amount of Rehydration/Reaction Buffer (blue cap)

   PCR Mix, 300 µL per portion of 25 reactions
3. Add the appropriate amount of Water (white cap) to Positive Control DNA, 600 µL
4. Incubate for 5 minutes at room temperature.
5. Vortex and centrifuge again.
6. Keep reagents on ice and store below -18 °C after rehydration.

Thermal Profile

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

Thermal Cycle Program

1 cycle	94 °C for 2 minutes
39 cycles	94 °C for 30 seconds
	55 °C for 30 seconds
	72 °C for 30 seconds
	cool down to 4–8 °C

The PCR Mastermix

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

1. The rehydrated PCR Mix can be prepared for 25 reactions, aliquoted as needed, and storedbelow –18 °C for up to 3 months. Aliquot 23 µL of the rehydrated PCR Mix into each PCR reaction tube.
2. Add 2 µL of deionized, DNA-free water as a negative control into negative control reaction tubes and sealto avoid contamination.
3. Add 2 µL of sample (as previously described) to PCR reaction tube per sample being tested and seal.
4. Pipette 2 µL of positive control DNA into positive control reaction tube.
5. Proceed to thermal cycling

Agarose Gel Run

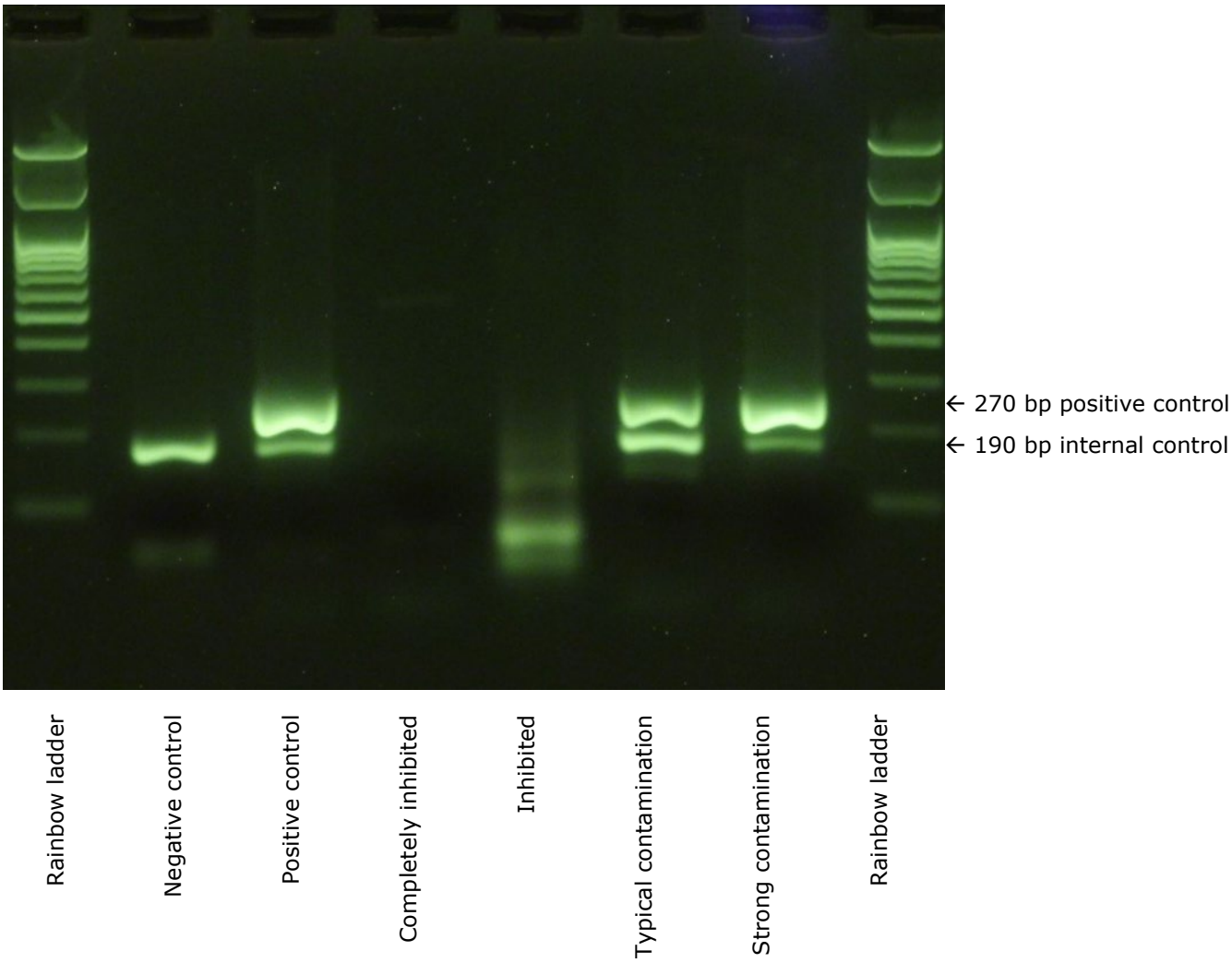
1. Use 1.5% standard agarose gel (A9539) with 5 mm comb.
2. Load 5 µL of each PCR reaction, mixed with gel tracking loading solution (G7654) per lane.
3. Stop electrophoresis after 2 cm run distance (depending on the electrophoresis chamber used e.g., run for 25 minutes at 100 V).

Results

Gel Evaluation

1. A distinct 190 bp band should appear in every lane indicating a successfully performed PCR. This band may fade out with increased amounts of amplicons formed, caused by mycoplasma DNA loads of 5×10^6 copies/ml.
2. No amplification of positive control DNA may be due to the following reasons:
   - positive control DNA tube has not been spun down before rehydration
   - programming mistake
   - pipetting mistake.
3. Before rerun of a negative and a positive control, please check thermocycler protocol and pipetting scheme.
4. Nonspecific bands on the gel are extremely rare. Possible primer self-annealing produces another (primer dimer) band of 80-90 bp but does not affect the precision or results of the test.
5. If the PCR of a sample is inhibited, PCR inhibitors can easily be removed from the sample by performing a DNA extraction with a commercially available kit (G1N10, G1N70, or NA2000).

Sample Data



Relevant amplicon sizes

Internal control	190 bp
Mycoplasma sp. (see Detectable Species)	~270 bp

Results of successfully performed PCR

PCR sample	Band pattern
Negative control	Band at 190 bp
Positive control	Band at 267 bp, possibly an additional band at 190 bp

Interpretation of possible band patterns

Band pattern	Interpretation
Band at 190 bp	Negative sample
Bands at 270 bp and 190 bp	Mycoplasma-positive sample with weak contamination
Strong band at 270 bp	Mycoplasma-positive sample with strong contamination
No band	PCR inhibition

Detectable Species

A large number of Mollicutes sequences have been published. The primers of the kit were aligned against the NCBI data and scrutinized for homologies within the target region of the 16S rRNA. At least 1 Ureaplasma, 7 Acholeplasma and 85 Mycoplasma show highly relevant sequence homologies and are presumably detected as positive.

Positive (Mollicutes)	Negative		
	EP 2.6.7 listed bacteria	Other microorganisms	Mammals
Acholeplasma laidlawii	*Clostridium acetobutylicum*	*Chlamydia trachomatis*	Vero-B4
Mycoplasma arginini	*Lactobacillus acidophilus*	*Legionella pneumophila*	Per.C6
Mycoplasma arthritidis	*Streptococcus pneumoniae*	*Micrococcus luteus*	RK13
Mycoplasma fermentans		*Candida albicans*	CHO-K1
Mycoplasma gallisepticum		*Enterococcus faecalis*	Murine genomic DNA
Mycoplasma genitalium		*Enterobacter aerogenes*	Calf thymus DNA
Mycoplasma hominis		*Escherichia coli Proteus*	Fetal bovine serum
Mycoplasma hyorhinis		*Proteus mirabilis*	
Mycoplasma orale		*Bacillus cereus*	
Mycoplasma penetrans			
Mycoplasma pneumoniae			
Mycoplasma salivarium			
Mycoplasma synoviae			
Spiroplasma citri			
Ureaplasma urealyticum			

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