

# GeneProof®

## Mycoplasma species

### PCR Kit



## in vitro Diagnostics

The kit is designed for professional use in specialized clinical and research laboratories.

### Method principles

This kit is designed for the detection of the genomic DNA in *Mycoplasma* species by the Polymerase Chain Reaction method (PCR). This detection is focused on a multi-copy sequence of the gene encoding bacterial 16S RNA, specific for *Mycoplasma* species. Sensitivity of the PCR detection kit runs in single copies of the *Mycoplasma* genomic DNA in a PCR reaction. It provides for a sensitive, universal detection of all clinically significant human mycoplasmas and ureaplasmas, including *M. pneumoniae*, *M. hominis*, *M. genitalium*, *U. urealyticum* and *M. fermentans*.

The kit is designed also for a sensitive detection of all *Mycoplasma* strains contaminating tissue cultures and solutions for cell cultures (e.g. virus cultures, BSA) and for the control of contamination in products prepared from cell cultures (e.g. vaccines). This method is uniquely adjusted for reaction inhibition detection and due to the simplicity of the whole detection process it provides standard results even during analysis of materials with high PCR inhibitor contents. This method is able to sensitively detect all known *Mycoplasma* strains (National Center for Biotechnology Information U.S.; 2006) and it is adjusted to detect at least the following strains of industrially or veterinary significant *Mycoplasma* species: *Acholeplasma axanthum*, *Mycoplasma alvi*, *Mycoplasma arginini*, *Mycoplasma buccale*, *Mycoplasma caripharngis*, *Mycoplasma cloacale*, *Mycoplasma fastidiosum*, *M. fermentans*, *Mycoplasma genitalium*, *Mycoplasma hominis*, *Mycoplasma salivarium*, *Mycoplasma pulmonis*, *M. orale*, *Mycoplasma penetrans*, *M. pirum*, *M. pneumoniae*, *Ureaplasma diversum*, *Ureaplasma parvum*, *Ureaplasma urealyticum*, *Acholeplasma* sp., etc.

The detection kit takes an advantage of the "hot start" technology, minimizing non-specific reactions and assuring maximum sensitivity. The reaction mix includes an internal standard controlling the possible inhibition of the PCR reaction and the uracil-DNA-glycosylase (UDG) controlling possible contamination of the PCR reaction by amplification products. This kit is designed for qualitative detection.

### Kit composition

	Cat. No. MSP/E/ISIN/025 25 reactions	Cat. No. MSP/E/ISIN/050 50 reactions	Cat. No. MSP/E/ISIN/100 100 reactions
<b>MasterMix</b> <i>Mycoplasma</i> species	1 x 900 µl	2 x 900 µl	4 x 900 µl
<b>Positive control</b> <i>Mycoplasma</i> species 10 <sup>2</sup> copies/µl	1 x 50 µl	1 x 50 µl	2 x 50 µl

### Storage and transportation conditions

Transport the kits at temperatures ranging from -20 °C to -80 °C. The kit remains stable for at least 6 months from the date of manufacturing at the temperature of -20°C. Repeated freezing and thawing of the MasterMix and the Positive control may result in lower detection quality. The manufacturer therefore recommends to aliquot the MasterMix by 36 µl directly to PCR tubes and hold in stock at -20°C. Positive control may be held in stock at 4°C.

### User Manual

#### Sampling and sample storage

Samples of body fluids, blood, vaginal and urethra swabs, peripheral blood, etc. are used for the *Mycoplasmas* detection. Sampling of all sample types, except for blood, should be performed into sterile tubes without any transportation media and the samples should be transported within 12 hours at +4°C. It is necessary to sample up to 2ml of body fluid samples or take wad smears or swabs "dry". **Blood sampling:** a sample of incoagulable peripheral blood should be sampled into the EDTA and transported into the laboratory at +4 °C within 24 hours. Sperm sampling is identical with the procedure for Chlamydia demonstration - there should be obtained one sample for the simultaneous demonstration of Chlamydia and Mycoplasmas (at least 200µl of ejaculate, transport at +4°C within 24 hours, otherwise store at min. -20°C until processing). In case of longer storage all samples should be frozen at -20°C.

#### DNA isolation

Isolation recommended by means of commercial DNA isolation kits according to the particular protocols of the isolation kit manufacturers. The manufacturer recommends the following isolation kits: PathogenFree DNA isolation kit (GeneProof); QIAamp DNA Blood Mini Kit (QIAGEN); NucleoSpin Blood (Macherey-Nagel).

### PCR amplification

1. Add **36 µl of the MasterMix** and **4 µl of the DNA isolate** or **4 µl of the Positive control** into the PCR tube. The final reaction mix volume should be 40 µl.\*
2. Insert the tubes into a thermocycler and amplify them according to the following recommended program:

**Amplification program: \*\***

UDG decontamination	37 °C/2 min.
initial denaturation	96 °C/15 min.
denaturation	96 °C/20 sec.
annealing	62 °C/20 sec.
extension	72 °C/40 sec.
number of cycles	45
final extension	72 °C/2 min.

\*Tubes should be maintained in cold environment when handled (at 0 – 15 °C)

\*\*Hereby presented amplification conditions may differ for various types of thermocyclers. The above listed conditions apply for PTC 200 (MJ Research) thermocycler.

### Agarose electrophoresis

The resulting amplification products should be separated on 2% agarose gel electrophoresis (5V/cm) containing Ethidium bromide (5 µg/1 ml) and visualized by means of UV illumination. Since the loading buffer is included in the reaction mix, there should be directly loaded at least 10 µl of the amplification product.

### Detection evaluation

**Positive sample** – is the detected amplification product with the length of 270 bp, there may also be the detected amplification product of the internal standard with the length of 603 bp (Fig. 1).

**Negative sample** – only the amplification product of the internal standard with the length of 603 bp is detected.

**Inhibiting sample** - should inhibition of the reaction components occur or should the reaction fail to proceed, none of the above-listed amplification products would be detected and the examination should be repeated.

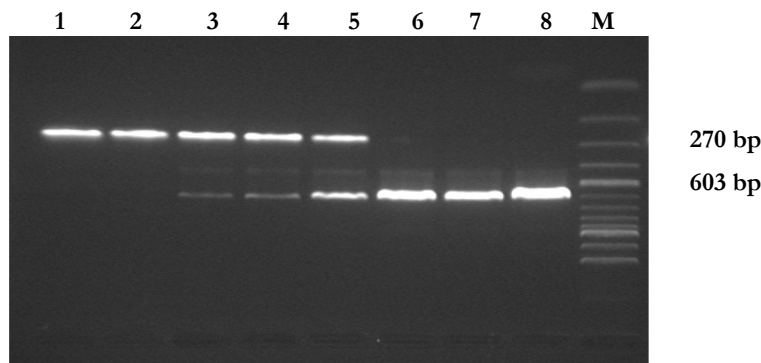


Fig. 1. Electrophoretical detection of amplification products on 2% agarose gel. **270 bp** long fragment results from the amplification of the target sequence of 16S rDNA *Mycoplasma* species, **603 bp** long fragment results from the amplification of the internal standard. Tracks No. 1 to 5 – results of the positive detection of *Mycoplasma species* in the sample, tracks No. 6 to 8 – result of negative detection, M – 100 bp weight marker.

### Warning:

- The kit is manufactured according to the European IVD Directive 98/79/EC.
- Be very careful when handling the Positive control or the clinical material – incorrect handling could result in contamination and the consequent impairment of the kit components or the MasterMix! The manufacturer is not responsible for the kit impairment due to incorrect handling.
- The kit should be disposed of after use according to the current legal regulations considering the fact that the kit doesn't contain any dangerous, infectious or toxic components that would be subject to special safety regulations and the packaging materials are made of paper and polypropylene.
- Service fee assuring returnability and recycling of the waste packaging material has been paid under the identification number EK-F00041495.

Manufacturer: GeneProof a.s.  
GeneProof a.s., Viniční 235, 615 00 Brno, Czech Republic  
Tel./Fax.: +420 543 211 679, e-mail: [office@geneproof.cz](mailto:office@geneproof.cz), [www.geneproof.cz](http://www.geneproof.cz)