

GeneProof®

Mycoplasma pneumoniae

PCR Kit



in vitro Diagnostics

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

Kit composition

Cat. No.	Internal Standard is included in the MasterMix for inhibition control			Contains a separate tube of Internal Standard for inhibition and isolation process control		
	MP/ISIN/025 25 reactions	MP/ISIN/050 50 reactions	MP/ISIN/100 100 reactions	MP/ISEX/025 25 reactions	MP/ISEX/050 50 reactions	MP/ISEX/100 100 reactions
MASTERMIX <i>Mycoplasma pneumoniae</i>	1 x 750 µl	2 x 750 µl	4 x 750 µl	1 x 750 µl	2 x 750 µl	4 x 750 µl
POSITIVE CONTROL <i>Mycoplasma pneumoniae</i> 10 ² copies/µl	1 x 200 µl	1 x 200 µl	2 x 200 µl	1 x 200 µl	1 x 200 µl	2 x 200 µl
INTERNAL STANDARD <i>Mycoplasma pneumoniae</i>	-	-	-	1 x 1000 µl	1 x 1000 µl	2 x 1000 µl

Storage and transportation conditions

Transport the kits at temperatures ranging from -20°C to -80°C. The kit remains stable for 9 months from the date of manufacturing at the temperature of -20°C. Repeated freezing and thawing of the MasterMix, Internal Standard or the Positive control may result in lower detection quality. The manufacturer therefore recommends to aliquot the MasterMix by 30 µl directly to PCR tubes and hold in stock at -20°C. Positive control and the Internal Standard may be held in stock at 4°C.

Pathogen information

M. pneumoniae causes 20-50% of all atypical pneumonias in common population worldwide. It is also believed to be a significant agent of community pneumonias, especially in school-age children and young people. There were reported cases of random occurrence, especially in elderly people and individuals with weakened immunity, though. Even though the infection is transmitted quite easily, bronchopneumonia develops in 3-20% cases only. The remaining infections proceed asymptotically or in the form of a rather light respiratory disease. The infection is predominantly transmitted by droplets through the air. The incubation period is reported to be 2-3 weeks and it may be (but doesn't have to be) accompanied by general symptoms of a respiratory infection. Symptoms are mostly demonstrated in the form of rising body temperature, weariness, coughing or the feelings of blocked nose. Atypical pneumonias caused by *M. pneumoniae* are characterized by poor physical and rich X-ray findings. If correct diagnosis is determined the Mycoplasma pneumonia therapy is usually without troubles and complications. In individuals with immunity disorders the infection may be rather serious, though. It may cause death in a rather short time. Mycoplasmas grow on special soils enriched with sterols and nucleic acid precursors. *M. pneumoniae* cultivations from nasopharynx swabs and sputum are not commonly performed because of technical demands and the maximum achieved detection rate of 60%. Indirect diagnostics are mostly used – serological demonstration of the infection (complement (KFR) bond and ELISA methods for antibody detection). In case of a commonly used KFR test an antibody titre of 1:64 is considered to be suspect; after 2 to 3 weeks it is necessary to make another sampling to evaluate the antibody creation dynamics. By means of the ELISA method it is possible to detect the IgA antibodies in about 1 week after the disease origination and IgM within 10 days. Polymerase Chain Reaction (PCR) is the most promising laboratory method of direct *M. pneumoniae* detection in clinical materials providing for quick and sensitive diagnostics of the disease.

Method principles

This kit is designed for *Mycoplasma pneumoniae* detection by the real-time Polymerase Chain Reaction (real-time PCR). The *M. pneumoniae* detection is based on the amplification of a specific conservative DNA sequence coding CARDS toxin and on measuring the amplification product concentration in the course of the PCR process by means of a fluorescence marked probe. *M. pneumoniae* presence is indicated by FAM fluorophore fluorescence growth. An Internal Standard (IS) is included in the reaction mix, controlling the possible inhibition of the PCR reaction or the efficiency of the DNA isolation process. IS positive amplification is detected in the fluorescence channel for the JOE fluorophore. The detection kit takes advantage of the "hot start" technology, minimizing non-specific reactions and assuring maximum sensitivity. It contains uracil-DNA-glycosylase (UDG), eliminating possible contamination of the PCR reaction by amplification products. The kit assures very high sensitivity of the laboratory *M. pneumoniae* detection in clinical material. The kit is designed for *in vitro* diagnostics and provides qualitative detection.

GeneProof PCR kits are designed for use with real-time devices from various manufacturers.

Mycoplasma pneumoniae PCR Kit has been validated with the following devices:

Rotor-Gene™ 3000 (Corbett Life Science)
Rotor-Gene™ 6000 (Corbett Life Science)
7500 Real-Time PCR System (Applied Biosystems)
LightCycler® 2.0 (Roche)
LightCycler® 480 System (Roche)
SLAN Real-time Quantitative PCR Fluorescent Detection System (Shanghai Odin Scienc & Technology Co.)

For detailed information about PCR kit use with specific devices see the Manufacturer's web site (www.geneproof.com) or request the information from your kit supplier.

If you want to use the kit with other real-time devices, contact the manufacturer, please: support@geneproof.com

Warning:

- The kit has been manufactured according to the EC Directive 98/79/EC as an *in vitro* medical diagnostic device.
- 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.

User Manual

Sampling and sample storage

Samples of sputum, BAL, nasopharyng swabs and nasal mucous membrane swabs are taken for *Mycoplasma pneumoniae* detection. Sampling of all sample types 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 about 1 ml of body fluid samples or take wad smears or swabs “dry”, without any media. In case of longer storage all samples should be frozen at -20°C.

DNA isolation

DNA isolation should be performed by isolation kits available at the market according to specific protocols for the particular microorganism isolation. The manufacturer recommends the following isolation kits: PathogenFree DNA Isolation Kit (GeneProof).

All GeneProof PCR kits include an Internal Standard (IS) providing for an effective monitoring of eventual inhibition of the PCR amplification and also of the isolation process efficiency. The Internal Standard is a precisely defined and quantified construct of a plasmid and insert, prepared by genetic engineering methods GeneProof develops and sells two basic variants of PCR kits which differ in the Internal Standard composition.

PCR Kit ISIN (Cat. No. MP/ISIN...)

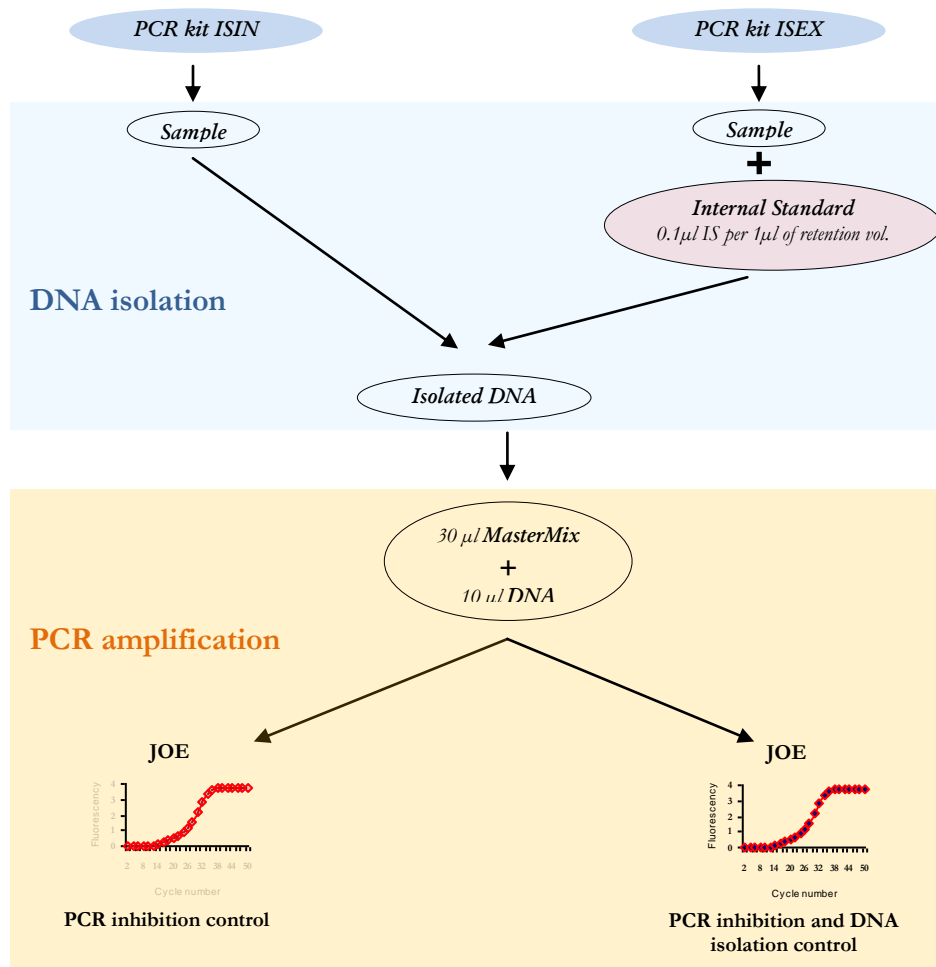
In this version of the PCR kit the Internal Standard (IS) is included directly in the MasterMix tube. This version of the kit provides **efficient control of the PCR reaction inhibition**.

PCR Kit ISEX (Cat. No. MP/ISEX...)

In this PCR kit version the Internal Standard (IS) is included in a separate tube within the package. This version of the PCR kit can be used for both **PCR reaction inhibition control** and **DNA isolation efficiency control**.

When using the ISEX versions of the PCR kits the IS should be added directly into the sample at the beginning of the isolation process so that in the end 1 µl of the resulting elution volume contains 0.1 µl of the IS:

Elution Volume	25 µl	50 µl	100 µl	200 µl
Internal Standard	2.5 µl	5 µl	10 µl	20 µl



PCR amplification

1. Add **30 µl of the MasterMix** and **10 µl of the DNA isolate** or **10 µl of the Positive Control** into a PCR tube. The final reaction mix volume should be 40 µl.
2. Close the tubes, centrifuge shortly, insert into the device and program according to the following table:

Amplification program:

UDG decontamination	37°C/2 min.
initial denaturation	95°C/10 min.
denaturation	95 °C/5 sec.
annealing	60°C/40 sec. - reading of the fluorescence signal
extension	72°C/20 sec.
number of cycles	45

Qualitative evaluation of detection

