

GeneProof®

Chlamydia pneumoniae

PCR Kit

CE

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		
	CHP/ISIN/025 25 reactions	CHP/ISIN/050 50 reactions	CHP/ISIN/100 100 reactions	CHP/ISEX/025 25 reactions	CHP/ISEX/050 50 reactions	CHP/ISEX/100 100 reactions
MASTERMIX <i>Chlamydia 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>Chlamydia 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>Chlamydia 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

Chlamydia pneumoniae is an intracellular respiration pathogen, causing mostly chronic bronchitis, „atypical“ pneumonias and sinusitis. Infections are common in the population and at the age of 50 years 70% of the population is seropositive. It is transmitted by air-borne infection and most of the population gets infected in childhood. Up to 70% of the infections are asymptomatic and they may recur during lifetime. Acute and chronic infections of the respiratory tract are related to the activation of the bronchial asthma. In elderly people this is one of the pathogens (besides *Haemophilus influenzae*, *Moraxella catarrhalis*, *Streptococcus pneumoniae* infection), causing serious chronic obstructive pulmonary diseases characterized by symptoms such as increased sputum volume, increased sputum purulence and dyspnoea. After the acute stage the respiratory tract infections frequently get chronic with persisting symptoms poorly reacting to ATB therapy. In children younger than 5 years *C. pneumoniae* infections are related to wheeziness and if neglected they may result in more serious respiratory diseases. Persisting *Chlamydias* are present even in the synovial fluid of patients with joint diseases. The infection is also a frequent complication of autoimmune eye and rheumatic diseases related to the presence of allele HLA-B27 (conjunctivitis, uveitis, reactive arthritis). Serological diagnostic method interpretation may be difficult due to high seroprevalence in population and a possible asymptomatic persistence. Therefore it is advantageous to use a direct detection of the infectious agents by the PCR method, detecting the pathogen in any clinical materials.

Method principles

This kit is designed for *Chlamydia pneumoniae* detection by the real time Polymerase Chain Reaction (real-time PCR). The *C. pneumoniae* detection is based on the amplification of a specific conservative DNA sequence of a single-copy *ompA* gene and on measuring the amplification product concentration in the course of the PCR process by means of a fluorescence marked probe. *C. 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 *C. 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.

Chlamydia 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, nasal mucous membrane swabs, eye swabs and tissues are taken for *Chlamydia pneumoniae* detection. Testing peripheral blood samples (*Chlamydia* occur in peripheral blood during infections) and synovial fluid in arthritic patients also yield precious information. 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 about 1 ml of body fluid samples or take wad smears or swabs "dry", without any media. Blood sampling: a sample of incoagulable peripheral blood should be sampled into DTA and transported to the laboratory at +4 °C within 24 hours. 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. CHP/ISIN...)

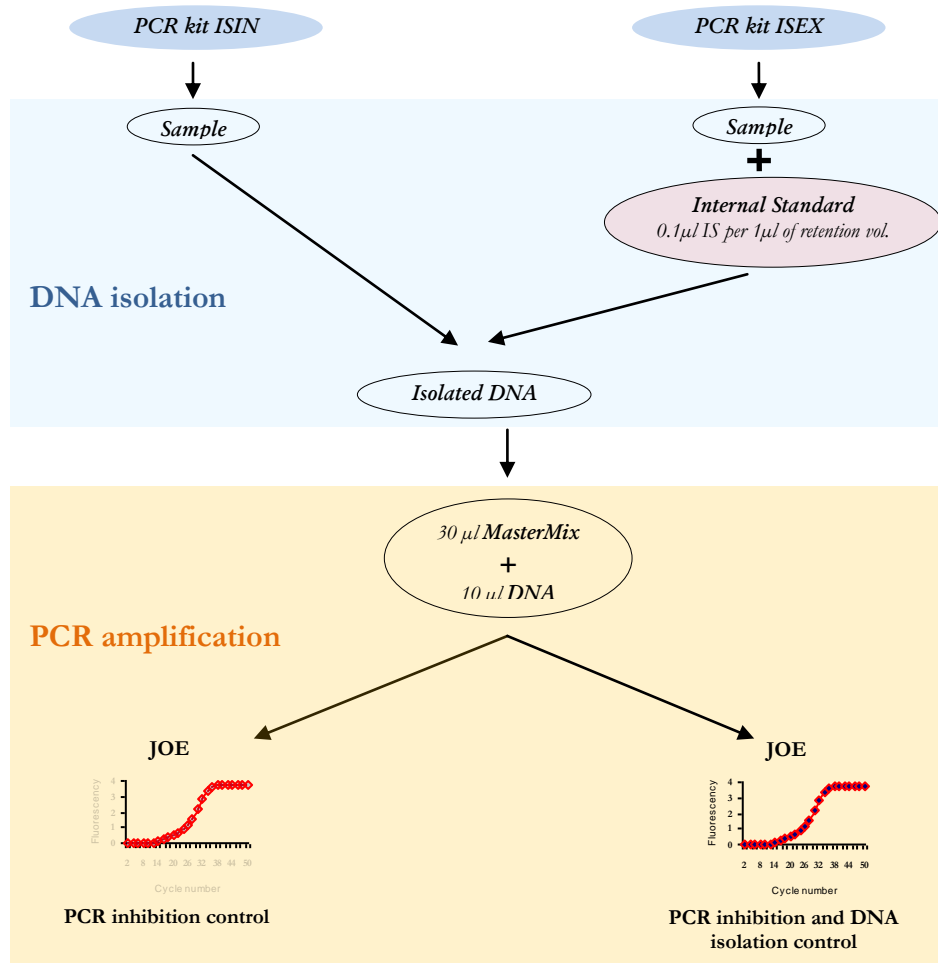
In this version of the PCR kit the Internal Standard (IS) is included directly in the MasterMix tube. This version of the kit provides **efficient control of the PCR reaction inhibition.**

PCR Kit ISEX (Cat. No. CHP/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

