

GeneProof®

Chlamydia pneumoniae

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 of *Chlamydia pneumoniae* by the Polymerase Chain Reaction method (PCR). This detection is aimed at a multi-copy sequence of the gene encoding the bacterial 16S RNA, specific for *Chlamydia pneumoniae*. The procedure takes an advantage of the “hot start” technology, minimizing non-specific reactions and assuring maximum sensitivity. An internal standard is included in the reaction mix, controlling the possible inhibition of the PCR reaction. Sensitivity of this method is 10¹ copies of *Chlamydia pneumoniae* genomic DNA per reaction. DNA isolation recommended by means of commercial kits designed for nucleic acid isolation.

Kit composition

	Cat. No. CHP/E/ISIN/025 25 reactions	Cat. No. CHP/E/ISIN/050 50 reactions	Cat. No. CHP/E/ISIN/100 100 reactions
MasterMix <i>Chlamydia pneumoniae</i>	1 x 900 µl	2 x 900 µl	4 x 900 µl
Positive control <i>Chlamydia pneumoniae</i> 10 ² 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 distribute 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 sputum, BAL, nasopharynx swabs, nasal mucous membrane swabs, eye scrapings and tissues are taken for *Chlamydia pneumoniae* detection. Testing peripheral blood samples (chlamydias 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 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. **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.*

(Note: MasterMix remains stable for at least 6 months at the temperature of -20°C. Repeated freezing and thawing of the MasterMix may result in lower detection quality. The manufacturer therefore recommends to distribute the MasterMix by 36 µl directly to PCR tubes and hold in stock at -20°C.

2. Insert the tubes into a thermocycler and amplify them according to the following recommended program:

Amplification program: **

initial denaturation	96°C/15 min.
denaturation	96°C/20 sec.
annealing	58°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

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

3. **Detection evaluation.** The resulting amplification products should be detected on 2% agarose gel electrophoresis 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. In case of positivity the detected amplification product's length will be 259 bp and the internal standard's length 464 bp (Fig. 1). Only amplification product of the internal control with the length of 464 bp is detectable in the negative sample. Should inhibition of the reaction components occur or should the reaction fail to proceed, there would be detectable none of the above-listed amplification products.

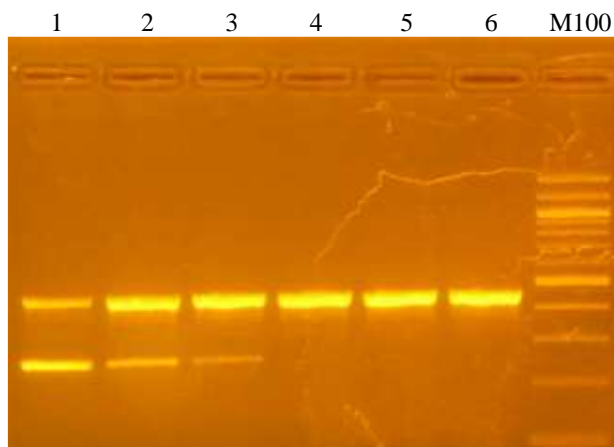


Fig. 1. Agarose electrophoresis result. 259 bp long fragment results from the amplification of the target sequence of 16S RNA *Chlamydia pneumoniae*, 464 bp long fragment results from the amplification of the internal standard. Track No. 1-3 represents the amplification of positive samples, No. 4-6 the amplification of negative samples, M-100 bp weight marker.

Warnings:

- 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 Master Mix! 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.

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