

GeneProof®

Chlamydia trachomatis

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 Enables PCR inhibition control			Internal Standard is included as an independent item Enables PCR inhibition control and DNA isolation process efficiency control.		
	CHT/ISIN/025 25 reactions	CHT/ISIN/050 50 reactions	CMV/ISIN/100 100 reactions	CHT/ISEX/025 25 reactions	CHT/ISEX/050 50 reactions	CHT/ISEX/100 100 reactions
MasterMix <i>Chlamydia trachomatis</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 trachomatis</i> 10 ² copies/µl	1 x 200 µl	1 x 200 µl	1 x 200 µl	1 x 200 µl	1 x 200 µl	1 x 200 µl
Internal Standard <i>Chlamydia trachomatis</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, the Positive Control and Internal Standard may result in lower detection quality. Positive Control may be held in stock at 4 °C.

Pathogen information

Chlamydia trachomatis is a common intracellular pathogen, able to infect mucosal surfaces of the urogenital system, rectum, nasopharynx and conjunctivae. These infections cause many diseases – nongonococcal urethritis, cervicitis, lymphogranuloma venereum, conjunctivitis, prostatitis and trachoma. This pathogen is one of the most frequent agents of sexually transmitted diseases (STDs). Serotypes D to K belong among the most frequent infection agents, causing inflammations in the urogenital system. These infections represent up to 50% of all urogenital infections in developed countries. In men it mostly causes the so-called non-blennorrhagic or post-blennorrhagic urethritis. In women they mostly affect the cervix and they are related to female sterility and cervix tumors. The infection may proceed without symptoms, though, avoiding diagnosis and facilitating transmission. Correct diagnosis is a basic prerequisite for effective treatment, consisting in administering antibiotics from the macrolide or tetracycline groups and quinolones. Chlamydia infection diagnostics is rather difficult due to the low sensitivity of the classic cultivation methods and antibody detection. Serological diagnostics yields little information. Therefore methods detecting this pathogen's DNA become the prime choice in the Chlamydia diagnostics. Highly sensitive and specific PCR method, which is generally 20-30% more sensitive than all diagnostic methods used so far, has been increasingly used for diagnosing urogenital and eye Chlamydia infections lately. This method is also very good for detecting Chlamydia contamination in sperm sampled for artificial insemination.

Method principles

The kit is designed for the detection of the *Chlamydia trachomatis* genomic and plasmidic DNA based on the amplification of the sequence of the gene for 16S RNA and concurrently on the amplification of the specific sequence of the *Ch. trachomatis* cryptic plasmid by means of the Polymerase Chain Reaction (PCR) and for measuring of the amplification product concentration growth in the course of the PCR by means of the fluorescence marked probe (real-time PCR).

The probe designated for pathogen detection is marked by the FAM fluorophore. The reaction mix includes an Internal Standard (IS) controlling the possible inhibition of the PCR reaction and the efficiency of DNA isolation process. Amplification of IS results in positive signal in JOE channel. The detection kit takes an advantage of the “hot start” technology, minimizing non-specific reactions and assuring maximum sensitivity and contains the uracil-DNA-glycosylase (UDG) controlling possible contamination of the PCR reaction by amplification products.

User Manual

Sampling and sample storage

To detect Chlamydia in case of urogenital and respiratory system infections it is necessary to perform scrapings with the aim to sample as much cellular material as possible (epithelia). In case of female urogenital system infections it is suitable to send for testing both the cervix scraping and the urethra scraping. Urethral sampling should be performed by a screw-like insertion of a tampon into the depth of 3-4 cm; the patient should not urinate in the course of the 2 hours preceding the sampling. The first 10-30ml of urine should be sampled for the urine testing (morning urine is not required). At least 200µl of ejaculate should be tested when establishing sperm Chlamydia contamination. Conjunctival sac fluid (or scraping) or cornea swab should be sampled for eye infection diagnostics. Sterility principles should be observed during sampling; samples should be placed into tubes without any transportation media and transported at 4 °C within 24 hours. In case of longer storage period freeze the sample to -20 to -80 °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); Arrow BUGS'n BEADS™ (NorDiag).

All GeneProof PCR kits include an Internal Standard 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 versions of PCR kits with various compositions of the Internal Standard:**

PCR kit ISIN (Cat. No. CHT/ISIN/...)

In this version of the PCR kit the Internal Standard is included in the MasterMix tube. This PCR kit version enables PCR inhibition control.

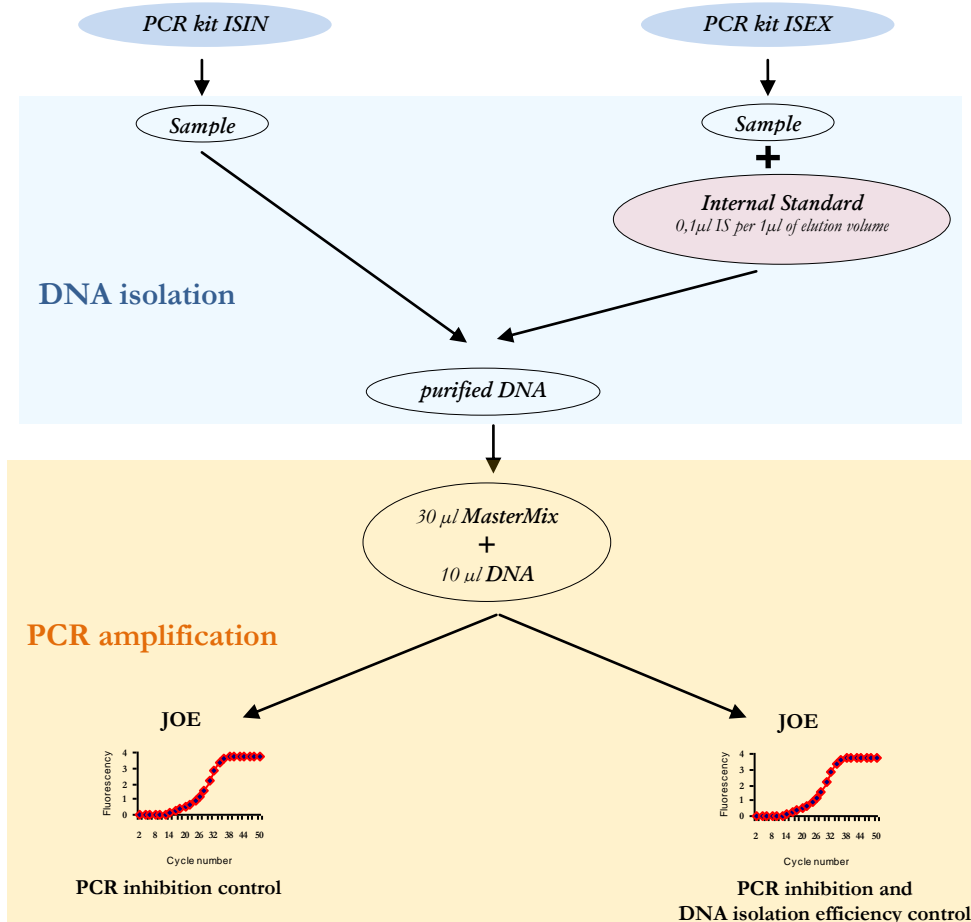
PCR kit ISEX (Cat. No. CHT/ISEX/...)

In this PCR kit version the Internal Standard is included as an independent item within the package. This PCR kit enables both, PCR inhibition control and DNA isolation process efficiency control.

The Internal Standard should be added into the sample at the beginning of the isolation process as follows:

0.1 µl of the Internal Standard per 1 µl of elution volume:

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 μ l of the MasterMix and 10 μ l of the DNA or 10 μ l of the Positive Control into a tube. The final reaction mix volume should be 40 μ l.
2. Close the tubes, shortly centrifuge, insert into the device and program according to the following table:

Amplification program:

UDG decontamination	37 °C/2 min.
initial denaturation	95 °C/15 min.
denaturation	96 °C/10 sec.
annealing	60 °C/60 sec. - reading of the fluorescence signal
extension	72 °C/40 sec.
number of cycles	45

GeneProof PCR kits are designed to be performed on real-time instruments of different manufacturers.

With following real-time instruments Chlamydia trachomatis PCR Kit was validated:

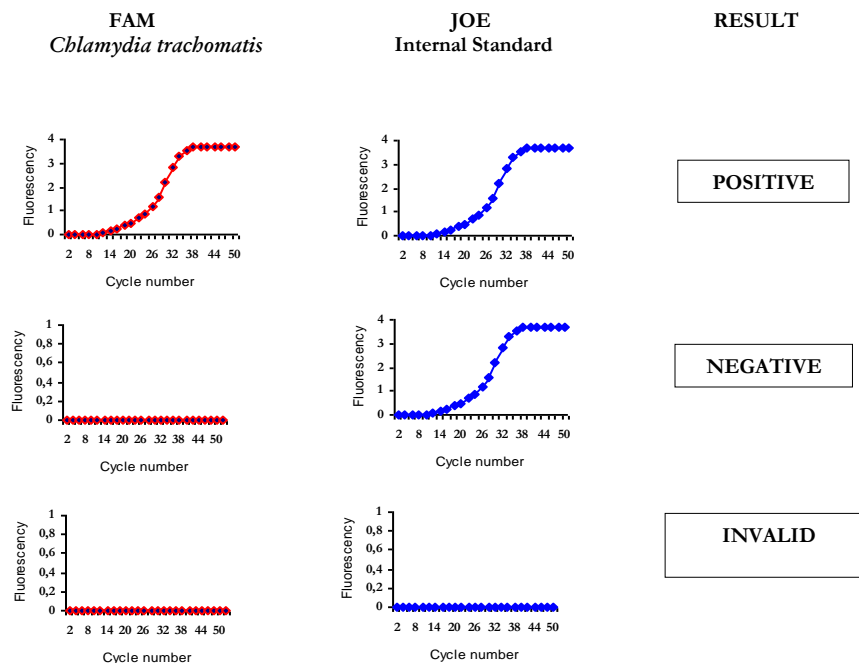
Rotor-Gene™ 3000 (Corbett Life Science)
 Rotor-Gene™ 6000 (Corbett Life Science)
 Mx3005P® QPCR System (Stratagene)
 Mx3000P® QPCR System (Stratagene)
 7500 Real-Time PCR System (Applied Biosystems)
 7300 Real-Time PCR System (Applied Biosystems)
 LightCycler® 2.0 (Roche)
 LightCycler® 480 System (Roche)
 SLAN Real-time Quantitative PCR Fluorescent Detection System (Shanghai Odin Scienc & Technology Co.)

Ask distributor of the kits for detailed manuals for the particular real-time devices or download them from the www.geneproof.com.

If you want use kit with other instrument mentioned above, contact please our Product Support Department at: support@geneproof.com

For information about using the PCR kits and assessing their results see the detailed manuals supplied by the manufacturer with the individual Real Time devices.

Qualitative evaluation of detection



Warning:

- The kit has been manufactured in harmony with 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.