GeneProof
Molecular diagnostics for your routine

GeneProof Chlamydia trachomatis PCR Kit

CE 1023 IVD

In vitro diagnostic medical device

The kit has been manufactured according to EC Directive 98/79/EC as an *in vitro* diagnostic medical device and it has been designed for professional use in specialized clinical and research laboratories.

KIT CONTENT

	ISIN Version IS included in the MasterMix			ISEX Version IS supplied in a separate tube Nucleic acid extraction and PCR inhibition control		
REF	**CHT/ISIN/025** 25 rxn	**CHT/ISIN/050** 50 rxn	**CHT/ISIN/100** 100 rxn	**CHT/ISEX/025** 25 rxn	**CHT/ISEX/050** 50 rxn	**CHT/ISEX/100** 100 rxn
MasterMix *Chlamydia trachomatis*	1x750 µl	2x750 µl	4x750 µl	1x750 µl	2x750 µl	4x750 µl
Positive Control *Chlamydia trachomatis*	1x200 µl	1x200 µl	2x200 µl	1x200 µl	1x200 µl	2x200 µl
Internal Standard *Chlamydia trachomatis*	-	-	-	1x1000 µl	1x1000 µl	2x1000 µl

STORAGE AND TRANSPORTATION CONDITIONS

The kit could be transported at temperature below -20 °C. The kit will remain stable at least until the expiry date printed on the package, if the storage temperature is kept (-20 ± 5 °C). After the first use, the kit is stable for maximum 5 repeated freezing/thawing cycles or for 14 days after the first use (whichever comes first).

TECHNICAL SPECIFICATION

Target Sequence	the cryptic plasmid multi-copy sequence and the 16S rRNA gene
Analytical Specificity	*Chlamydia trachomatis*, including mutations with deletions in cryptic plasmid (including Swedish variant), 100 %
Analytical Sensitivity (LoD)	reaches up to 0.075 cp/µl with the probability of 95 % (on Amplirun® *Chlamydia trachomatis* DNA control, Vircell)
Diagnostic Specificity	99.21% ($CI_{95\%}$: 99.07% - 99.34%)
Diagnostic Sensitivity	100% ($CI_{95\%}$: 99.58% - 100%)
Validated Specimen	sperm, swab, urine
External Quality Assessment	regularly tested by QCMD and Instand e.V. External Quality Assessment Panels
Regulatory Status	CE_{1023} IVD

Quality management system is certified in compliance with the requirements of the standard ČSN EN ISO 13485 ed.2:2016

INTERFERENCES

The interferences testing was performed using negative urine spiked with CT positive control at 3x LoD. The urine samples were obtained from healthy donors and tested values of interfering substances were spiked into well-characterized urine samples. Varying levels of bilirubin, urea, uric acid, albumin, low pH, high pH level and D-glucose in samples have been tested in the presence and absence of CT DNA. All tests were performed using one lot of GeneProof CT assay. The pathological levels of all markers for testing have been set according to the literature, hospital recommendations and guidelines (http://www.southend.nhs.uk/media/180421/pf_biochemistry_reference_intervals.pdf) and recommendations of Czech Society of Clinical Biochemistry.

Substance tested	Levels tested	Interference observed	Substance tested	Levels tested	Interference observed
Albumin	5 %	Partial	pH	Acidic condition (pH 4)	None
Albumin	Routine level	None	pH	Basic condition (pH 9)	None
Bilirubin	1 % (w/v)	Partial	Uric acid	5 mmol/L	Partial
Bilirubin	0,5 % (w/v)	None	Uric acid	0,4 mmol/L; 1,4mmol/L	None
Glucose	0,1 % (w/v); 1 % (w/v)	None	Uric acid	Routine level	None

The results indicate no interference with respect to CT assay sensitivity, however, the partial inhibition was recognized based on missing of internal standard.

METHOD PRINCIPLES

The PCR kit is designed for *Chlamydia trachomatis* detection by the real-time Polymerase Chain Reaction (PCR) method. The *C. trachomatis* detection consists in amplification of both the cryptic plasmid multi-copy sequence and the 16S rRNA gene specific for *C. trachomatis* and in measurement of fluorescence increase. Detection of multi-copy sequence of the cryptic plasmid enables very high sensitivity of *Chlamydia* detection (including the Swedish variant) and the chromosomal gene detection at the same time enables high specificity and makes detection of plasmid-less strains possible. The *C. trachomatis* presence is indicated by the FAM fluorophore fluorescence growth. An Internal Standard (IS) is either included in the reaction mix, controlling the possible inhibition of the PCR (ISIN version) or excluded, controlling also the DNA extraction process quality (ISEX version). IS positive amplification is detected in the HEX fluorophore fluorescence channel. The detection kit takes advantage of the "hot start" technology, minimizing non-specific reactions and assuring maximum sensitivity. Ready to Use MasterMix contains uracil-DNA-glycosylase (UDG), eliminating possible contamination of the PCR by amplification products. The kit is designed for *in vitro* diagnostics and provides qualitative detection.

ISIN version

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

ISEX version

Internal Standard is provided as independent item within the package. This PCR kit version enables both PCR inhibition control and nucleic acid purificaion process efficiency control.

USER MANUAL

SAMPLING AND SAMPLE STORAGE

Swabs and scrapings - urethral and cervical sampling should be performed by a screw-like insertion of a swab (DacronR, Rayon, etc) into the depth of 3-4 cm; the patient should not urine for 2 hours preceding the sampling. Urine - the first 10-30 ml of urine into a sterile tube without any transportation media should be sampled for urine testing. Centrifuge urine sample (at least 1 ml) at 11 000 rpm for 5 minutes before DNA extraction. Sperm - sampling into a sterile tube without any transportation media or spermicide substances should be performed 2 to 3 days after any previous ejaculation. Preservation and transport - tubes without any transportation media; the samples should be preserved at the temperature between +2 °C and +8 °C and transported within 24 hours. In case of longer storage period freeze the samples to the temperature below -10 °C.

NUCLEIC ACID PURIFICATION

Nucleic acid extraction should be performed by extraction kits available at the market according to protocols for the particular clinical material extraction. The manufacturer recommends the following extraction kits:

GeneProof PathogenFree DNA Isolation Kit
croBEE NA16 Nucleic Acid Extraction System

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 SETUP

1. Add 30 µl of MasterMix into PCR tubes.

2. Add 10 µl of the isolated nucleic acid sample or 10 µl of Positive Control into the individual PCR tubes. The final reaction mix volume will be 40 µl. It is necessary to keep all components at +2 °C to +8 °C during the PCR preparation. The customer has to use his own negative control in the form of water, buffer or isolate of negative clinical material in each test.

3. Close the tubes, centrifuge shortly, insert them into the device and let them amplify according to the following PCR profile.
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! The manufacturer is not responsible for the kit impairment due to incorrect handling.

AMPLIFICATION PROFILE

Step	Temperature	Time	Data Collection	Cycles
Hold	37 °C	2 min		1
Hold	95 °C	10 min		1
PCR	95 °C	5 s	FAM+HEX	45
	60 °C	40 s		
	72 °C	20 s		

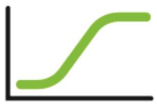
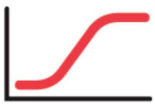
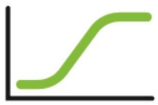
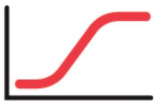
INSTRUMENTS

GeneProof Chlamydia trachomatis PCR Kit is designed for use with real-time devices from various manufacturers:

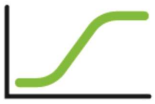
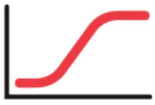
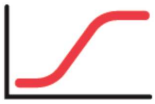
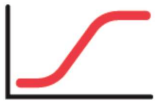
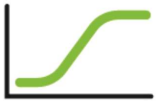
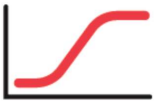
croBEE Real-Time PCR System
Applied Biosystems 7300 / 7500 Real-Time PCR System
AriaMx Real-Time PCR System
CFX Connect™ / CFX96™/ Dx Real-Time PCR Detection System
DTlite Real-Time PCR System
LightCycler® 2.0 / 480
LineGene 9600 / 9600 Plus
Rotor-Gene 3000 / 6000 / Q
SLAN® Real-Time PCR System
StepOneTM Real-Time PCR System

Required Channels: FAM, HEX.
GeneProof diagnostic kits are continually verified with various types of devices. Current list is available at www.geneproof.com or request the list at support@geneproof.com.

CLINICAL SAMPLES ANALYSIS EVALUATION

Channel FAM	Channel HEX	Result	Interpretation	
		**Valid**	*Chlamydia trachomatis*	**positive**
		**Valid**	*Chlamydia trachomatis*	**positive**
		**Valid**	*Chlamydia trachomatis*	**negative**
		**Invalid**		

CONTROL ANALYSIS EVALUATION

	Channel FAM	Channel HEX			Result
Positive Control			or		**Valid**
Negative Control			or		**Valid**
Positive Control			or		**Invalid**
Negative Control			or		**Invalid**

WARNING

A single valid Instruction For Use for a specific kit is included in the package or to be requested for the particular lot from the manufacturer. 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. If you have any questions please contact our Customer Service.

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Orders
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