GeneProof Chlamydia pneumoniae PCR Kit

CE 1023 IVD

In vitro diagnostic medical device

The kit has been manufactured according to the 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

REF:	CHP/ISEX/025 25 rxn	CHP/ISEX/100 100 rxn
MasterMix *Chlamydia pneumoniae*	1 x 750 µl	4 x 750 µl
Positive Control *Chlamydia pneumoniae*	1 x 200 µl	2 x 200 µl
Internal Control *Bordetella* *Chlamydia pneumoniae* *Mycobacterium tuberculosis* *Mycoplasma pneumoniae*	1 x 1000 µl	2 x 1000 µl

STORAGE AND TRANSPORTATION CONDITIONS

The kit must 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. The components are stable for a maximum of 5 repeated freezing / thawing cycles after the first use of a particular vial. The component must be used before the expiry date or 30 days after the first use of a particular vial (whichever comes first).

TECHNICAL SPECIFICATION

Target Sequence	specific conservative DNA sequence of a single-copy *ompA* gene
Analytical Specificity	*Chlamydia pneumoniae,* 100 %
Analytical Sensitivity (LoD)	0.647 cp/µl with the probability of 95 % (on Amplirun® *Chlamydia pneumoniae* DNA control, Vircell)
Diagnostic Specificity	100 % ($CI_{95\%}$: 95.01 % - 100.00 %)
Diagnostic Sensitivity	99.08 % ($CI_{95\%}$: 94.26 % - 99.95 %)
Validated Specimen	BAL, sputum, swab
External Quality Assessment	regularly tested in 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 interfering endogenous substances have been tested for routine concentration level in nasopharyngeal swabs. According to the CLSI EP07-A2 and CLSI MM3-A3 the proposed interferent substances are blood in the concentration of 2 %, mucin in the concentration 60 µl/ml and human DNA in concentration 1-2000 µg/l. Blood was reported as an inhibitor if it was present at 4 % or more in the reaction volume (≥4 µl of blood/100 µl reaction mixture). It is recommended to keep blood below 2 % of the 100 µl reaction volume (Kern et al, 2009). Mucins (glycoproteins) as well as complex polysaccharides, were described as potential PCR inhibitors (Schrader et al, 2012).

SWAB

Tested substance	Tested level(s)	Observed interference	Tested substance	Tested level(s)	Observed interference
Whole blood	2 % (v/v)	None	Mucin	60 µg/ml	None
Sodium chloride	6.5 mg/ml	None	Oxymetazoline	0.0625 mg/ml	None
Xylometazoline	0.25 mg/ml	None	Phenylephrine	0.375 mg/ml	None
Budesonidum	100 µg	Partial	Neomycin, bacitracin	300 I.U. (neomycin) 25 I.U. (bacitracin)	None

BAL

Tested substance	Tested level(s)	Observed interference	Tested substance	Tested level(s)	Observed interference
Whole blood	2 % (v/v)	None	Mucin	60 µg/ml	None

METHOD PRINCIPLE

The PCR kit is designed for *Chlamydia pneumoniae* detection by the real-time Polymerase Chain Reaction (PCR) method. The *C. pneumoniae* detection consists in amplification of a specific conservative DNA sequence of a single-copy *ompA* gene and in measurement of fluorescence increase. The *C. pneumoniae* presence is indicated by the FAM fluorophore fluorescence growth. The Internal Control (IC) is controlling the possible inhibition of the PCR and also the DNA extraction process quality. IC 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 Master Mix 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.

USER MANUAL

SAMPLING AND SAMPLE STORAGE

Samples of sputum, bronchoalveolar lavage (BAL) and swab are taken for the *Chlamydia 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 the temperature (2 – 8) °C. It is necessary to sample about 1 ml of body fluid samples or take wad smears or swabs "dry". In case of longer storage keep all samples frozen at 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 products:

GeneProof PathogenFree DNA Isolation Kit

croBEE 201A Nucleic Acid Extraction Kit

Add the Internal Control (IC) directly into the sample at the beginning of the extraction process, so that in the end 1 µl of the resulting elution volume contains 0.1 µl of the IC:

Elution volume	**25 µl**	**50 µl**	**100 µl**	**200 µl**
Internal Control	2.5 µl	5 µl	10 µl	20 µl

PCR SETUP

1. Gently vortex and briefly centrifuge the Master Mix and Positive Control tubes.

2. Add 30 µl of Master Mix into PCR tubes.

3. Add 10 µl of the isolated nucleic acid sample or 10 µl of Positive Control into the individual PCR tubes and mix by pipetting. The total reaction mix volume will be 40 µl. *It is necessary to keep all components at (2 – 8) °C during the PCR preparation. The isolate of negative isolation control with Internal Control should be used in each test. The negative clinical material, water or buffer can be used as negative isolation control. The customer must use his own negative control.*

4. 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		45
	60 °C	40 s	FAM + HEX	
	72 °C	20 s		

INSTRUMENTS

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

croBEE Real-Time PCR System
AMPLilab Real-Time PCR System
Applied Biosystems 7300 / 7500 Real-Time PCR System
AriaMx Real-Time PCR System
BioQuant-96 Real-Time PCR System
CFX Connect™ / CFX96™/ Dx Real-Time PCR Detection System
LightCycler® 2.0 / 480
LineGene 9600 / 9600 Plus
Mic qPCR Cycler
QuantStudio™ 3 / 5 Real-Time PCR System
Rotor-Gene 3000 / 6000 / Q
SLAN® Real-Time PCR System / StepOne™ 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 can be requested at *support@geneproof.com*

CLINICAL SAMPLE ANALYSIS EVALUATION

Chanel FAM (CP)	Channel HEX (IC)	Result	Interpretation
+	+	Valid	*C. pneumoniae* positive
+	-	Valid	*C. pneumoniae* positive
-	+	Valid	*C. pneumoniae* negative
-	-	Invalid	-

CONTROL ANALYSIS EVALUATION

	Signal	Channel	Run Validity	Recommendation
Positive Control	+	FAM	Valid	-
Positive Control	-		Invalid	Repeat PCR run
Negative control	-		Valid	-
Negative control	+		Invalid	Repeat PCR run

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. Use only combination of components from the specific PCR kit. 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 care.

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