Package insert

GeneProof
Molecular diagnostics for your routine

GeneProof Neisseria gonorrhoeae PCR Kit

CE 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 isolation and PCR inhibition control		
REF	**NG/ISIN/025** 25 rxn	**NG/ISIN/050** 50 rxn	**NG/ISIN/100** 100 rxn	**NG/ISEX/025** 25 rxn	**NG/ISEX/050** 50 rxn	**NG/ISEX/100** 100 rxn
MasterMix *Neisseria gonorrhoeae*	1x750 µl	2x750 µl	4x750 µl	1x750 µl	2x750 µl	4x750 µl
Positive Control *Neisseria gonorrhoeae*	1x200 µl	1x200 µl	2x200 µl	1x200 µl	1x200 µl	2x200 µl
Internal Standard *Neisseria gonorrhoeae*	-	-	-	1x1000 µl	1x1000 µl	2x1000 µl

STORAGE AND TRANSPORTATION CONDITIONS

The kits should be transported and stored at temperatures between -85 °C and -10 °C. The kit will remain stable at least until the expiry date printed on the package, if the storage temperature is kept. Repeated freezing and thawing of the kit components may result in lower detection quality.

TECHNICAL SPECIFICATION

Target sequence	conservative DNA region of the single-copy *porA* pseudo-gene and the DNA conservative region encoding 16S rRNA
Specificity	*N. gonorrhoeae* including mutants in the *porA* pseudo-gene region
Sensitivity (LOD)	reaches 0.219 genome/ µl with the probability of 95 %
Sample types	urine, urethra, rectum and cervix swabs, sperm
Quality Control	regularly tested by QCMD and Instand e.V. External Quality Assessment Panels

METHOD PRINCIPLES

The PCR kit is designed for *Neisseria gonorrhoeae* detection by the real-time Polymerase Chain Reaction (PCR) method. The *Neisseria gonorrhoeae* detection is based on the amplification of a multi-copy sequence of the gene encoding 16S rRNA and *porA* pseudogene specific for *Neisseria gonorrhoeae* and measuring the amplification product concentration using the PCR process and fluorescence labelled probes. *Neisseria gonorrhoeae* 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 (ISIN version) and possibly 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 reaction by amplification products. The kit performs very sensitive *Neisseria gonorrhoeae* detection in clinical material (urine, urethral, cervical and rectal swab, sperm). The kit is designed for *in vitro* diagnostics and provides qualitative detection.

The kit can sensitively detect even recently discovered *porA* mutants of *Neisseria gonorrhoeae* that may go undetected by other kits.

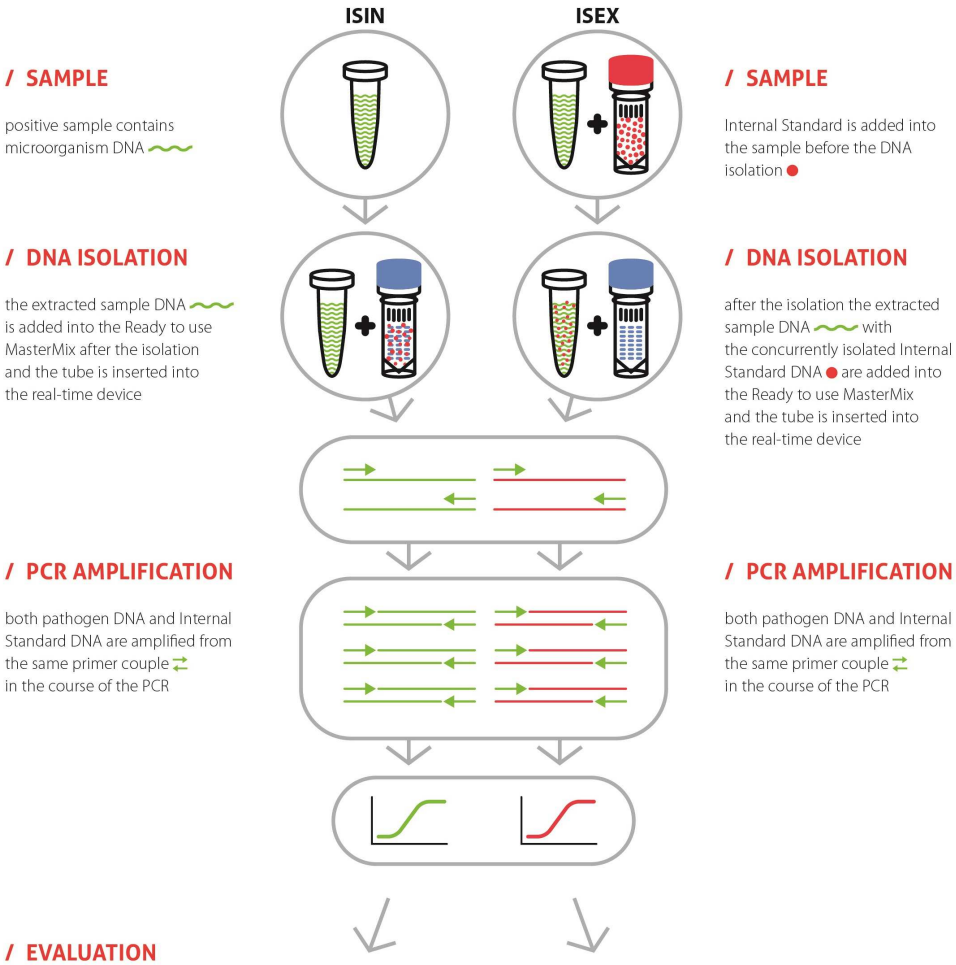
ISIN version

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.

MICROBIOLOGICAL DNA DETECTION TECHNOLOGY



USER MANUAL

SAMPLING AND SAMPLE STORAGE

Sampling of all samples, especially of swabs (swabs from urethra, vagina, cervix, prostate exprimate) should be performed "dry" into sterile tubes without any transportation media and the samples should be transported within 24 hours at the temperature between +2 °C and +8 °C. Detection in urine samples (sample at least 2 ml just like for the *Chlamydia* diagnostics) is possible but problematic due to the usually rather low and fluctuating gonococci concentrations. Rare disseminated gonococci infections and arthritis can be detected in the samples of incoagulable peripheral blood (in EDTA) and synovial fluid. In case of longer storage keep all samples frozen at the temperature between -85 °C and -10 °C.

NUCLEIC ACID PURIFICATION

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

GeneProof PathogenFree DNA Isolation Kit
croBEE NA16 Nucleid 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.

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 or the MasterMix! The manufacturer is not responsible for the kit impairment due to incorrect handling.

AMPLIFICATION PROFILE

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

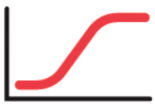
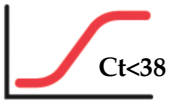
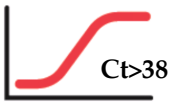
VALIDATED INSTRUMENTS

GeneProof PCR kits are designed for use with real-time devices from various manufacturers. This PCR kit has been validated with the following devices:

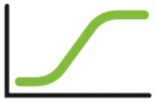
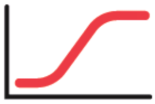
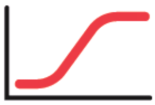
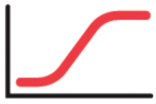
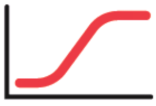
Applied Biosystems 7300/7500 Real-Time PCR System
AriaMx Real-Time PCR System
Dx/CFX96™/CFX Connect™ Real-Time PCR Detection System
LightCycler ® 2.0, LightCycler ® 480
LineGene 9600
Mx3000P/3005P QPCR System
Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q
SLAN® Real-Time PCR System
StepOne™ Real-Time PCR System

GeneProof diagnostic kits are continually validated with various types of devices. Please request the current list at support@geneproof.com.

CLINICAL SAMPLES ANALYSIS EVALUATION

Channel FAM	Channel HEX	Result	Interpretation	
		**Valid**	*Neisseria gonorrhoeae*	**positive**
		**Valid**	*Neisseria gonorrhoeae*	**positive**
	 Ct<38	**Valid**	*Neisseria gonorrhoeae*	**negative**
	 Ct>38	**Invalid**		
		**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 package insert 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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