

# GeneProof®

## *Neisseria gonorrhoeae*

### 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 for inhibition control			Contains a separate tube of Internal Standard for inhibition and isolation process control		
	NG/ISIN/025 25 reactions	NG/ISIN/050 50 reactions	NG/ISIN/100 100 reactions	NG/ISEX/025 25 reactions	NG/ISEX/050 50 reactions	NG/ISEX/100 100 reactions
<b>MASTERMIX</b> <i>Neisseria gonorrhoeae</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
<b>POSITIVE CONTROL</b> <i>Neisseria gonorrhoeae</i> 10 <sup>2</sup> copies/µl	1 x 200 µl	1 x 200 µl	2 x 200 µl	1 x 200 µl	1 x 200 µl	2 x 200 µl
<b>INTERNAL STANDARD</b> <i>Neisseria gonorrhoeae</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, Internal Standard or the Positive control may result in lower detection quality. The manufacturer therefore recommends to aliquot the MasterMix by 30 µl directly to PCR tubes and hold in stock at -20°C. Positive control and the Internal Standard may be held in stock at 4°C.

## Pathogen information

Gonorrhoea is a serious disease from the group of classic sexually transmitted diseases. It is caused by the pathogenic bacteria *Neisseria gonorrhoeae* (gonococcus) and it is characteristic by a usually non-invasive inflammation of urogenital mucous membranes. Clinically it is manifested in both men and women as a urethritis with purulent discharge from urethra, in women also as a cervicitis. Infection in pelvic region may be a complication which may result in infertility in women. There is a significant percentage of asymptomatic carriers. Penicillin-based antibiotics commonly used for therapy are still quite effective yet there is an increasing number of resistant strains. Concurrent Chlamydia infection is also frequent. Cultivation methods are dominant in the gonorrhoea diagnostics but their sensitivity is frequently negatively influenced by the improper transportation of samples into the laboratory due to the high sensitivity of the gonococci to temperature variations and also due to gonorrhoea self-treatment attempts by means of incorrectly used antibiotics. In case of the cultivation detection failure there is usually used the comparatively little-sensitive microscopic diagnostics. Methods demonstrating the presence of nucleic acids have been increasingly used even in the gonorrhoea diagnostics. The gene probe method is currently a classic approach but PCR methods have started to dominate due to their higher sensitivity. These methods are commonly used in the gonorrhoea diagnostics for the direct detection of gonococci in any clinical materials. This method is suitable for gonococci detection in the samples from vaginal swabs, cervix swabs, male and female urethra swabs and in specific cases also in rectum and larynx swabs. It is necessary to take into account the up to 3-week persistence of PCR positivity in the mucous membrane swab samples. Therefore a follow-up examination after a treatment should be performed 3-4 weeks after the end of the treatment. High sensitivity of this method provides for gonococci detection also in urine samples, eye scrapings and in case of disseminated infections also in blood and synovial fluid.

## Method principles

The kit is designed for the detection of the *Neisseria gonorrhoeae* genomic DNA based on the amplification of a part the gene encoding the *porA* pseudogene 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 kit is designed for amplification and for subsequent qualitative detection in the Rotor-Gene™ device. *N. gonorrhoeae* DNA presence in the sample is indicated by the FAM fluorophore fluorescence growth. An Internal Standard (IS) is included in the reaction mix, controlling the possible inhibition of the PCR reaction and the efficiency of the DNA isolation process. IS positive amplification is detected in the fluorescence channel for the JOE fluorophore. The detection kit takes an advantage of the “hot start” technology, minimizing non-specific reactions and assuring maximum sensitivity. It contains uracil-DNA-glycosylase (UDG), eliminating possible contamination of the PCR reaction by amplification products. This assures very high sensitivity of the laboratory *N. gonorrhoeae* detection in clinical material. The kit is designed for *in vitro* diagnostics and provides qualitative detection.

**GeneProof PCR kits are designed for use with real-time devices from various manufacturers.**

***Neisseria gonorrhoeae* PCR Kit has been validated with the following devices:**

**Rotor-Gene™ 3000 (Corbett Life Science)**  
**Rotor-Gene™ 6000 (Corbett Life Science)**  
**7500 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.)**

For detailed information about PCR kit use with specific devices see the Manufacturer's web site ([www.geneproof.com](http://www.geneproof.com)) or request the information from your kit supplier.

If you want to use the kit with other real-time devices, contact the manufacturer, please: [support@geneproof.com](mailto:support@geneproof.com)

### Warning:

- The kit has been manufactured according to 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.

# 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 of +4°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 all samples should be frozen at -20°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).

To improve sensitivity it is recommended to increase concentration of the urine sample by centrifugation (14 000 to 18 000 g/ 15 to 20 min.), carefully remove the supernatant up to the amount entering isolation (usually 200 µl) and use this pre-processed sample for isolation. To improve the examination sensitivity it is recommended to use the smallest retention volume recommended by the isolation kit manufacturer (usually 50 µl).

All GeneProof PCR kits include an Internal Standard (IS) 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 variants of PCR kits which differ in the Internal Standard composition.

### PCR Kit ISIN (Cat. No. NG/ISIN...)

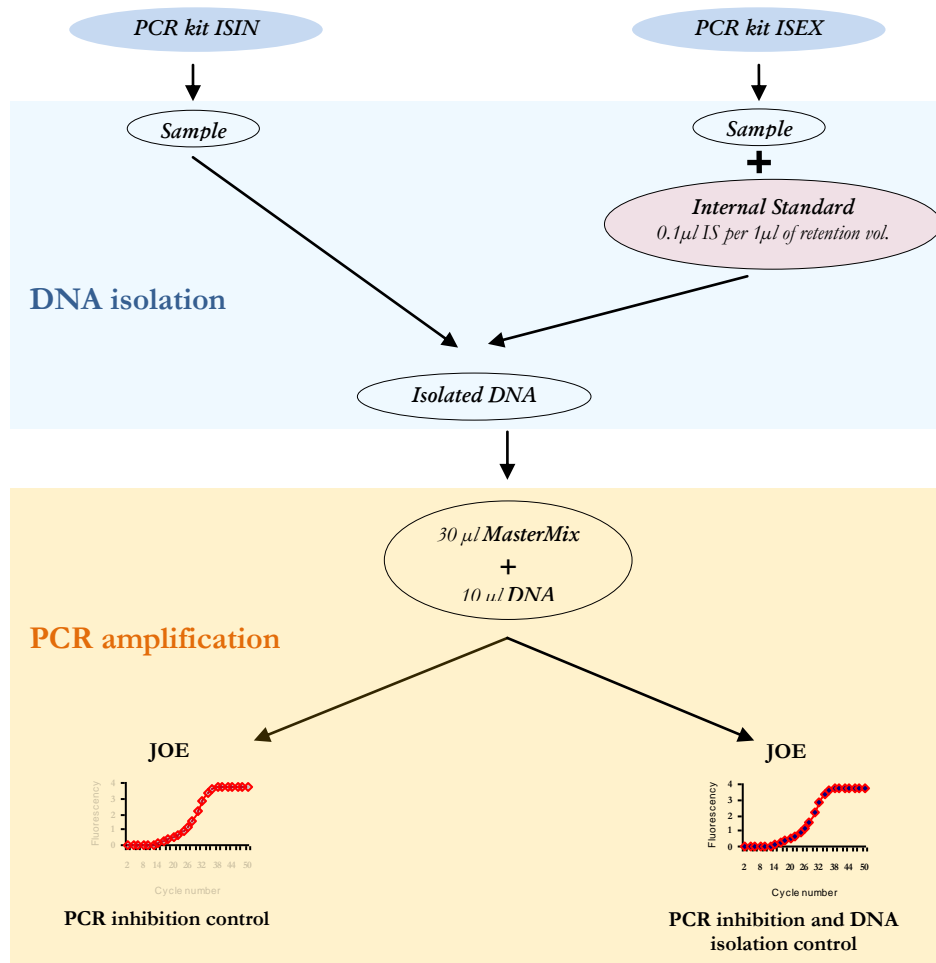
In this version of the PCR kit the Internal Standard (IS) is included directly in the MasterMix tube. This version of the kit provides **efficient control of the PCR reaction inhibition.**

### PCR Kit ISEX (Cat. No. NG/ISEX...)

In this PCR kit version the Internal Standard (IS) is included in a separate tube within the package. This version of the PCR kit can be used for both **PCR reaction inhibition control** and **DNA isolation efficiency control.**

**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 amplification

1. Add **30 µl of the MasterMix** and **10 µl of the DNA isolate** or **10 µl of the Positive Control** into a PCR tube. The final reaction mix volume should be 40 µl.
2. Close the tubes, centrifuge shortly, insert into the device and program according to the following table:

### Amplification program:

UDG decontamination	37°C/2 min.
initial denaturation	95°C/10 min.
denaturation	95 °C/5 sec.
annealing	60°C/40 sec. - reading of the fluorescence signal
extension	72°C/20 sec.
number of cycles	45

## Qualitative evaluation of detection

