



For *in Vitro* Diagnostic Use

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**AmpliSens[®] *Neisseria gonorrhoeae*-
screen-FRT PCR kit**

Instruction Manual



Ecoli s.r.o., Studenohorská 12
841 03 Bratislava 47
Slovak Republic
Tel.: +421 2 6478 9336
Fax: +421 2 6478 9040
ecoli@ecoli.sk
www.ecoli.sk www.pcrdiagnostics.eu



Federal State Institution of Science
Central Research Institute of Epidemiology
3A Novogireevskaya Street
Moscow 111123 Russia

1. INTENDED USE.

AmpliSens® Neisseria gonorrhoeae-screen-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Neisseria gonorrhoeae* DNA in the clinical materials (scrapes (swabs) of urogenital tract mucous membranes; throat, anorectal, and conjunctival swabs; urine sediment; secret of the prostate gland; synovial fluid) by means of real-time hybridization-fluorescence detection.

2. PRINCIPLE OF PCR DETECTION.

Neisseria gonorrhoeae detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using special *Neisseria gonorrhoeae* primers. In real-time PCR the amplified product is detected via fluorescent dyes. These dyes are usually linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. The real-time PCR monitoring of the fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening of the reaction tubes after the PCR run. **AmpliSens® Neisseria gonorrhoeae-screen-FRT** PCR kit is a qualitative test, which contains the Internal Control (IC). It must be used in the isolation procedure in order to control the isolation process of each individual sample and to identify possible reaction inhibition. PCR kit uses “hot-start”, which greatly reduces frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by separation of nucleotides and Taq-polymerase by using wax layer or chemically modified polymerase (TaqF). Wax melting and reaction components mix occur only at 95 °C. Chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

3. CONTENT.

AmpliSens® Neisseria gonorrhoeae-screen-FRT PCR kit is produced in 3 forms:

AmpliSens® *Neisseria gonorrhoeae*-screen-FRT PCR kit variant FRT (for use with RG) **REF** R-B51(RG)-CE.

AmpliSens® *Neisseria gonorrhoeae*-screen-FRT PCR kit variant FRT (for use with iQ) **REF** R-B51(iQ)-CE.

AmpliSens® *Neisseria gonorrhoeae*-screen-FRT PCR kit variant FRT-100 F (for use with RG,iQ) **REF** R-B51-F(RG, iQ)-CE.

AmpliSens® Neisseria gonorrhoeae-screen-FRT PCR kit, variant FRT includes:

Reagent	Description	Volume (ml)	Amount
PCR-mix-1-FEP/FRT Neisseria gonorrhoeae-screen ready-to-use single-dose test tubes (<i>under wax</i>)	colorless, clear liquid	0.008	110 tubes
PCR-mix-2-FL	colorless, clear liquid	0.77	1 tube
Positive Control DNA Neisseria gonorrhoeae (C+)	colorless, clear liquid	0.2	1 tube
DNA-buffer	colorless, clear liquid	0.5	1 tube
Negative Control (C-)*	colorless, clear liquid	1.2	1 tube
Internal Control-FL (IC)**	colorless, clear liquid	1.0	1 tube

* must be used in the isolation procedure as Negative Control of Extraction.

** add 10 µl of Internal Control-FL during the DNA isolation procedure directly to the sample/lysis mixture (see “DNA-sorb-AM” **REF** K1-12-100-CE or “DNA-sorb-B”, **REF** K1-2-100-CE protocols).

AmpliSens® *Neisseria gonorrhoeae*-screen-FRT PCR kit is intended for 110 reactions, including controls.

AmpliSens® Neisseria gonorrhoeae-screen-FRT PCR kit, variant FRT-100 F includes:

Reagent	Description	Volume (ml)	Amount
PCR-mix-1-FEP/FRT Neisseria gonorrhoeae-screen	colorless, clear liquid	1.2	1 tube
PCR-mix-2-FRT	colorless, clear liquid	0.3	2 tubes
Polymerase (TaqF)	colorless, clear liquid	0.06	1 tube
Positive Control DNA Neisseria gonorrhoeae (C+)	colorless, clear liquid	0.2	1 tube
DNA-buffer	colorless, clear liquid	0.5	1 tube
Negative Control (C-)*	colorless, clear liquid	1.2	1 tube
Internal Control-FL (IC)**	colorless, clear liquid	1.0	1 tube

* must be used in the isolation procedure as Negative Control of Extraction.

** add 10 µl of Internal Control-FL during the DNA isolation procedure directly to the sample/lysis mixture (see “DNA-sorb-AM” **REF** K1-12-100-CE or “DNA-sorb-B”, **REF** K1-2-100-CE protocols).

AmpliSens® *Neisseria gonorrhoeae*-screen-FRT PCR kit is intended for 110 reactions, including controls.

4. ADDITIONAL REQUIREMENTS.

- DNA isolation kit
- Disposable powder-free gloves and laboratory coat
- Pipettes (adjustable)
- Sterile pipette tips with aerosol barriers (up to 200 µl)

- Tube racks
- Vortex mixer
- Desktop centrifuge with rotor for 2 ml reaction tubes
- PCR box
- Rotor-Gene™ 3000 or Rotor-Gene™ 6000 (Corbett Research, Australia) Instrument; iQ5 or iQ iCycler (BioRad, USA) Instrument
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity (for example, “Axygen”, USA).
- Refrigerator from 2 to 8 °C
- Deep-freezer with temperature below minus 16°C.
- Waste bin for used tips.

5. GENERAL PRECAUTIONS.

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers and use new tip for every procedure.
- Store extracted positive material (samples, controls and amplicons) away from all other reagents and add it to the reaction mix in a separate area.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use a kit after its expiration date.
- Dispose of all specimens and unused reagents in accordance with local regulations.
- Specimens should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.
- Avoid sample or reagent contact with the skin, eyes and mucose membranes. If any of these solutions come into contact, rinse immediately with water and seek medical advice immediately.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- Workflow in the laboratory must proceed in a one directional manner, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents to the area in which the previous step was performed.



Some components of this kit contain Sodium Azide as a preservative. Do not use metal tubing for reagent transfer.

6. SAMPLING AND HANDLING.



Obtaining of biological materials samples for PCR-analysis, transportation, and storage are described in manufacturer’s handbook [1]. It is recommended that this handbook is read before starting work.

AmpliSens® Neisseria gonorrhoeae-screen-FRT PCR kit is intended for the analysis of DNA extracted with DNA isolation kits from:

- *scrapes (swabs) of urogenital tract mucous membranes;*
- *throat swabs;*
- *anorectal swabs;*
- *conjunctival swabs;*

- *urine sediment (use the first portion of a morning urine specimen);*
- *secret of the prostate gland;*
- *synovial fluid.*

7. PROTOCOL.

7.1. DNA Isolation

It’s recommended that the following nucleic acid extraction kits are used:

- “DNA-sorb-AM”, **REF** K1-12-100-CE.
- “DNA-sorb-B”, **REF** K1-2-100-CE (for the secret of the prostate gland).



Carry out the DNA isolation according to the manufacturer’s instructions.

7.2. Preparing the PCR.

Total reaction volume is **25 µl**, the volume of DNA sample is **10 µl**.

7.2.1 Preparing tubes for PCR.

Variant FRT.

1. Collect the required number of the tubes with **PCR-mix-1-FEP/FRT Neisseria gonorrhoeae-screen** and wax for amplification of DNA from clinical and control samples.
2. Add **7 µl** of **PCR-mix-2-FL** to the surface of wax layer of each tube, so that it does not fall under the wax and mix with **PCR-mix-1-FEP/FRT Neisseria gonorrhoeae-screen**.

Variant FRT-100 F.

1. Collect the required number of the tubes for amplification of DNA of clinical and control samples (0.2 ml tubes for 36-Well rotor or 0.1 ml stripes for 72-Well rotor).
2. For performing N reactions (including 2 controls) mix in a new tube: **10*(N+1) µl of PCR-mix-1-FEP/FRT Neisseria gonorrhoeae-screen**, **5.0*(N+1) µl of PCR-mix-2-FRT** and **0.5*(N+1) µl of polymerase (TaqF)**. Vortex the tube, then centrifuge shortly. Transfer **15 µl** of prepared mix into each tube. Steps 3 and 4 are applied for both variants.
3. Using tips with aerosol barrier add **10 µl** of **DNA samples** obtained from clinical or control samples at the stage of DNA extraction into prepared tubes.



The tubes with **PCR-mix-1-FEP/FRT Neisseria gonorrhoeae-screen** that are not used at the moment should be kept away from light.

4. Carry out control amplification reactions:

NCA Add **10 µl** of **DNA-buffer** to the tube labeled NCA (Negative Control of Amplification).

C+ Add **10 µl** of **Positive Control DNA Neisseria gonorrhoeae** to the tube labeled C+ (Positive Control of Amplification).

7.2.2. Amplification.

7.2.2.1. RG.

1. Program the Rotor-Gene™ according to manufacturer's manual and Appendix 1.
2. Create a temperature profile on your Rotor-Gene™ instrument as follows:

AmpliSens-1 RG program

Step	Temperature, °C	Time	Fluorescence detection	Cycle repeats
Hold	95	15 min	–	1
Cycling	95	5 sec	–	5
	60	20 sec	–	
	72	15 sec	–	
Cycling 2	95	5 sec	–	40
	60	20 sec	FAM/Green, JOE/Yellow	
	72	15 sec	–	

3. Fluorescence detection is on the 2-nd pass (**60°C**) in FAM/Green and JOE/Yellow fluorometer channels.
4. Make the adjustment of the fluorescence channel sensitivity according to Appendix 1.



It is possible to use **AmpliSens-1 RG** general program that allows simultaneous conducting of any combination of tests for detection of sexually transmitted diseases pathogens DNA including tests for identifying of *Human Papillomaviruses* by means of AmpliSens HPV HCR PCR kits.



If “multiprime” format tests for detection of sexually transmitted diseases (AmpliSens PCR kits) are carried out simultaneously, the program and template corresponding to those “multiprime” tests should be applied.

7.2.2.2. iQ

1. Program the iQ™ according to manufacturer's manual and Appendix 2.
2. Create a temperature profile on your iQ™ instrument as follows:

AmpliSens-1 iQ program

Step	Temperature, °C	Time	Fluorescence detection	Cycle repeats
Hold	95	15 min	–	1
Cycling	95	5 sec	–	5
	60	20 sec	–	
	72	15 sec	–	
Cycling 2	95	5 sec	–	40
	60	30 sec	FAM-490, HEX-530	
	72	15 sec	–	

3. Fluorescence detection in fluorometer channels, FAM and HEX, is on the 2-nd pass (**60°C**).
4. Make the adjustment of the fluorescence channel sensitivity according to Appendix 2.



It is possible to use **AmpliSens-1 iQ** general program that allows simultaneous conducting of any combination of tests for detection of sexually transmitted diseases pathogens DNA including tests for identifying of *Human Papillomaviruses* by means of AmpliSens HPV HCR PCR kits.

8. DATA ANALYSIS.

RG. Internal Control is detected on the JOE/Yellow fluorescence channel, *Neisseria gonorrhoeae* DNA is detected on the FAM /Green fluorescence channel.

See **Appendix 1** for data analysis settings for Rotor-Gene™ 3000 or Rotor-Gene™ 6000.

iQ. Internal Control is detected on the HEX fluorescence channel, *Neisseria gonorrhoeae* DNA is detected on the FAM fluorescence channel.

See **Appendix 2** for data analysis settings for iQ5 or iQiCycler.

8.1. Results interpretation.

The results are interpreted with the software of Rotor-Gene™ 3000 or Rotor-Gene™ 6000 Instrument or iQ5 or iQiCycler Instrument by the crossing (or not) of the fluorescence curve with the threshold line.

Results are accepted as relevant if both positive and negative controls of amplification along with negative control of extraction are passed.

Results for controls

Control	Stage for control	Ct in channel		Interpretation
		FAM /Green	JOE/Yellow/HEX	
C-	DNA isolation	Neg	Pos (< X*)	OK
NCA	Amplification	Neg	Neg	OK
C+	Amplification	Pos (< Z*)	Neg	OK

*For X, Y values see Appendix 1 in case of using Rotor-Gene™ 3000 or Rotor-Gene™ 6000 Instrument or Appendix 2 in case of using iQ5 or iQiCycler Instrument.

1. The sample is considered as positive for *Neisseria gonorrhoeae* if its Ct value is defined in the results grid on FAM/Green channel.
2. The sample is considered as negative for *Neisseria gonorrhoeae* if its Ct value is not defined in the results grid (the fluorescence curve does not cross the threshold line) on FAM/Green channel and in the results grid on the JOE/Yellow/HEX channel the Ct value doesn't exceed X.

9. TROUBLESHOOTING.

If analysis results are not obtained as per the following examples:

1. PCR and detection of the samples should be repeated, if Ct values are absent on FAM/Green and JOE/Yellow channel or Ct value on JOE/Yellow channel exceed X. In case the same result is obtained once again, it is necessary to repeat the sample analysis, starting from the extraction stage.
2. If positive result (fluorescence curve crosses the threshold line) is registered for the sample that has fluorescence curve without typical exponential growth (graph is linear), it can suggest about incorrect threshold line setting or incorrect calculation of base line parameters. Such a result should not be considered as positive. If threshold line was set correctly, the PCR should be repeated for the sample.

(This option is applicable only for iQ Instruments)

- If no signal is detected for Positive Controls of amplification, it can suggest of incorrect programming of the temperature profile of the Rotor-Gene™ 3000 or Rotor-Gene™ 6000 Instrument, incorrect configuration of the PCR reaction, or storage conditions for kit components has not complied with manufacturer instruction, or the reagents kit has expired. It is necessary to ensure adequate programming of the Rotor-Gene™ Instrument (see 7.2.2.1.), storage conditions, and check the expiration date of the reagents, and then repeat PCR reaction once again.
- If signal is registered in Negative Control of Extraction (C-) on FAM/Green channel and/or in Negative Control of amplification (NCA) on any of the channels, it indicates the contamination of reagents or samples. In this case results of the analysis for all samples are considered invalid. It is required to repeat the analysis of all tests, and also to take measures to detect and eliminate the source of contamination.

If you have any further questions or if you encounter problems, please contact our Authorized representative in the European Community.

10. STABILITY AND STORAGE.

All components of the **AmpliSens® Neisseria gonorrhoeae-screen-FRT** PCR kit (except for Polymerase(TaqF) and PCR-mix-2-FRT) are to be stored at 2-8 °C when not in use. All components of the **AmpliSens® Neisseria gonorrhoeae-screen-FRT** PCR kit are to be stable until the labeled expiration date.



Polymerase (TaqF) and PCR-mix-2-FRT are to be stored not more than minus 16 °C.

11. SPECIFICATIONS.

11.1. Sensitivity.

Analytical Sensitivity of **AmpliSens® Neisseria gonorrhoeae-screen-FRT** PCR kit is no less than 1×10^3 genome equivalents per 1 ml of sample (GE/ml).



The claimed analytical features of **AmpliSens® Neisseria gonorrhoeae-screen-FRT** PCR kit are guaranteed only when additional reagents kits “DNA-sorb-AM” or “DNA-sorb-B” (manufactured by Federal State Institution of Science Central Research Institute of Epidemiology) are used.

11.2. Specificity.

Specificity of **AmpliSens® Neisseria gonorrhoeae-screen-FRT** PCR kit is assured by selection of specific primers and probes, as well as the selection of strict reaction conditions. The primers and probes were checked for possible homologies to all in gene banks published sequences by sequence comparison

analysis. Specificity of **AmpliSens® Neisseria gonorrhoeae-screen-FRT** PCR kit was confirmed in laboratory clinical trials.

















12. REFERENCES.

- Handbook “Sampling, transportation, storage of clinical material for PCR diagnostics”, developed by Federal State Institution of Science “Central Research Institute of Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow, 2008.

13. QUALITY CONTROL.

In compliance with Federal State Institution of Science “Central Research Institute of Epidemiology” ISO 13485 – certified Quality Management System, each lot of **AmpliSens® Neisseria gonorrhoeae-screen-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

14. EXPLANATION OF SYMBOLS.

	Manufacturer		Temperature limitation
	Use by		Batch code
	For <i>in Vitro</i> Diagnostic Use		Version
	Catalogue number		Internal Control complex
	Contains sufficient for <n> tests		Authorized representative in the European Community.
	Consult instructions for use		Caution, consult accompanying documents
	For working with Rotor-Gene™ 3000/6000		For working with iQ5, iQ iCycler
	Positive control		Negative control