ECOLI

eSens

CE

IVD

For Professional Use Only

eSens N.gonorrhoeae /M.genitalium QL PCR kit

REF ES3049A

Instructions for Use

1 INTENDED USE

eSens N.gonorrhoeae /M.genitalium QL PCR kit is an *in vitro* nucleic acid amplification test for simultaneous detection of *Neisseria gonorrhoeae* and *Mycoplasma genitalium* DNA in the clinical materials (urogenital, rectal, and oropharyngeal swabs; conjunctival discharge; prostate gland secretion; and urine samples) by using real-time hybridization-fluorescence detection of amplified products.

NOTE: The results of PCR analysis are taken into account in complex diagnostics of disease.

2 PRINCIPLE OF PCR DETECTION

Neisseria gonorrhoeae and *Mycoplasma genitalium* detection by the multiplex polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific regions using specific primers. In real-time PCR, the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes that bind specifically to the amplified product during thermocycling. The real-time monitoring of the fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run.

eSens N.gonorrhoeae /M.genitalium QL PCR kit is a qualitative test that contains the Internal Control-FL (Internal Control-FL (IC)). It must be used in the extraction procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition.

eSens N.gonorrhoeae /M.genitalium QL PCR kit uses “hot-start”, which greatly reduces the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by separation of nucleotides and Taq-polymerase by using a wax layer or a chemically modified polymerase (TaqF). Wax melts and reaction components mix only at 95 °C. Chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

The PCR kit contains the system for prevention of contamination by amplicons using the enzyme uracil-DNA-glycosylase (UDG) and deoxyuridine triphosphate. The enzyme UDG recognizes and

catalyzes the destruction of the DNA containing deoxyuridine, but has no effect on DNA containing deoxythymidine. Deoxyuridine is absent in the authentic DNA, but is always present in amplicons, because deoxyuridine triphosphate is a part of dNTP mixture in the reagents for the amplification. Due to the deoxyuridine containing contaminating amplicons are sensitive to the destruction by UDG before the DNA-target amplification. So, the amplicons cannot be amplified.

The enzyme UDG is thermolabile. It is inactivated by heating at temperature above 50 °C. Therefore, UDG does not destroy the target amplicons which are accumulated during PCR.

The results of amplification are registered in the following fluorescence channels.

Table 1

Channel for fluorophore	FAM	ROX	Cy5
DNA-target	*N.gonorrhoeae*	*M.genitalium*	Internal Control-FL
Target gene	*16s rRNA* gene	*gyrB* gene	genetically engineered construction

3 CONTENT

Reagent	Description	Volume, ml	Quantity
PCR-mix-1-FL *N.gonorrhoeae / M.genitalium*	clear liquid from colorless to light lilac colour	1.2	1 tube
PCR-mix-2-FRT	colorless clear liquid	0.3	2 tubes
Polymerase (TaqF)	colorless clear liquid	0.03	2 tubes
Positive Control complex (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 extraction procedure as Negative Control of Extraction.

** add **10 µl** of **Internal Control-FL (IC)** during the DNA extraction procedure directly to the sample/lysis mixture

eSens N.gonorrhoeae /M.genitalium QL PCR kit is intended for 110 reactions (including controls).

4 ADDITIONAL REQUIREMENTS

- Transport medium.
- DNA extraction kit.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol filters (up to 100 µl) in tube racks.

- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 2 ml reaction tubes.
- PCR box.
- Real-time instruments (for example, Rotor-Gene Q (QIAGEN, Germany), CFX 96 Touch, CFX 96 Opus (Bio-Rad, USA), QuantStudio 5 (Thermo Fisher Scientific), or equivalent).
- Disposable polypropylene PCR tubes (0.1- or 0.2-ml):
    - 0.2-ml PCR tubes with optical transparent domed or flat caps if a plate-type instrument is used;
    - 0.2-ml PCR tubes with flat caps or strips of four 0.1-ml Rotor-Gene PCR tubes if a rotor-type instrument is used.
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir for used tips.

5 GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers and use a new tip for every procedure.
- Store all extracted positive material (specimens, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable protective gloves and laboratory cloths, and protect eyes while samples and reagents handling. 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.
- Samples should be considered potentially infectious and handled in biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all samples or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
- Avoid samples and reagents contact with the skin, eyes, and mucous membranes. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice immediately.
- Safety Data Sheets (SDS) are available on request.
- Use of this product should be limited to personnel trained in DNA amplification techniques.
- Workflow in the laboratory must be one-directional, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where 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

eSens N.gonorrhoeae /M.genitalium QL PCR kit is intended for analysis of DNA extracted by using DNA extraction kits from the clinical material (urogenital swabs; rectal swabs; oropharyngeal swabs; conjunctival discharge and prostate gland secretion; urine samples (use the first part of the stream)).

7 WORKING CONDITIONS

eSens N.gonorrhoeae /M.genitalium QL PCR kit should be used at 18–25 °C.

8 PROTOCOL

8.1 DNA extraction

Any commercial nucleic acid extraction kit, if IVD-CE validated for the indicated specimen types, could be used.

Ecoli Dx, s.r.o. recommends:

- For the manual extraction

- **DNA-sorb-AM** (K1-12-100-CE)

- For the automatic extraction

- **ePure STD DNA Extraction Kit** (E2007)

The DNA extraction of each test sample is carried out in the presence of **Internal Control-FL (IC).**

NOTE: Extract DNA according to the manufacturer's protocol.

8.2 Preparing PCR

8.2.1 Preparing tubes for PCR

The type of tubes depends on the PCR instrument used for analysis. Use disposable filter tips for adding reagents, DNA and control samples into tubes.

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

1. Thaw the required number of the tubes with **PCR-mix-2-FRT.** Vortex the tubes with **PCR-mix-1-FL *N.gonorrhoeae/M.genitalium*, PCR-mix-2-FRT,** and **polymerase (TaqF)** and then centrifuge briefly.

   Take the required number of tubes/strips for amplification of the cDNA obtained from clinical and control samples.

2. For N reactions (including 2 controls), add to a new tube:

   10*(N+1) µl of PCR-mix-1-FL *N.gonorrhoeae / M.genitalium*,

   5.0*(N+1) µl of PCR-mix-2-FRT

   0.5* (N+1) µl of polymerase (TaqF).

   Vortex the tube, then centrifuge shortly. Transfer **15 µl** of the prepared mixture to each tube.

3. Using tips with aerosol filter, add **10 µl** of **DNA** obtained at the DNA extraction stage to the prepared tubes.
4. Carry out the 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 complex** to the tube labeled C+ (Positive Control of Amplification).

C–	Add **10 µl** of **the sample extracted from the Negative Control reagent** to the tube labeled C– (Negative control of Extraction).

8.2.2 Amplification

1. Create a temperature profile on your instrument as follows:

Table 2

eSens-1 amplification program

	Rotor-type instruments (For example, Rotor-Gene Q (QIAGEN, Germany) or equivalent.)			**Plate-type instruments** (For example, CFX 96 (Bio-Rad, USA) or equivalent.)		
Step	Temperature, °C	Time	Cycles	Temperature, °C	Time	Cycles
1	95	15 min	1	95	15 min	1
2	95	5 s	5	95	5 s	5
	60	20 s		60	20 s	
	72	15 s		72	15 s	
3	95	5 s	40	95	5 s	40
	60	20 s Fluorescence acquiring		60	30 s Fluorescence acquiring	
	72	15 s		72	15 s	

Fluorescent signal is detected in the channels for the FAM, ROX and Cy5 fluorophores.

2. Adjust the fluorescence channel sensitivity.
3. Insert tubes into the reaction module of the device.
4. Run the amplification program with fluorescence detection.
5. Analyze results after the amplification program is completed.

8.3 Instrument Settings

Test settings for rotor-type instruments

Channel	Calibrate/Gain Optimisation	Threshold	Dynamic tube	Slope Correct	More Settings/ Outlier Removal
FAM/Green	from 5 FI to 10 FI	0.1	On	Off	0%
ROX/Orange	from 4 FI to 8 FI	0.1	On	Off	5%
Cy5/Red	from 4 FI to 8 FI	0.07	On	On	5-10%

Test settings for plate-type instruments

Note - Set ***Ramp Rate* 2,5 °C/s** by clicking the *Step Options* button for each step of cycling.

Channel	Threshold
FAM, ROX, Cy5	For each channel in *Log* Scale set the threshold line at the level of 10-20 % of maximum fluorescence obtained for the Positive Control of Amplification (C+) in the last amplification cycle.

9 DATA ANALYSIS

Analysis of results is performed by the software of the real-time PCR instrument used by measuring fluorescence signal accumulation in four channels:

- The signal of the *Neisseria gonorrhoeae* DNA amplification product is detected in the channel for the FAM fluorophore,
- The signal of the *Mycoplasma genitalium* DNA amplification product is detected in the channel for the ROX fluorophore.
- The signal of the IC DNA amplification product is detected in the channel for the Cy5 fluorophore.

Results are interpreted by the crossing (or not-crossing) the fluorescence curve with the threshold line set at the specific level that corresponds to the presence (or absence) of a *Ct* value of the cDNA sample in the corresponding column of the results grid.

Principle of interpretation is the following:

- ***Neisseria gonorrhoeae*** DNA is **detected** if the *Ct* value is determined in the results grid in the channel for the **FAM** fluorophore. Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.
- ***Mycoplasma genitalium*** DNA is **detected** if the *Ct* value is determined in the results grid in the channel for the **ROX** fluorophore. Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.
- ***Neisseria gonorrhoeae* and *Mycoplasma genitalium* DNA are not detected** in a sample if the *Ct* value is not determined (absent) in the channels for FAM and ROX fluorophores, whereas the *Ct* value determined in the channel for the Cy5 fluorophore is less than the boundary *Ct* value.
- The result is **invalid** if the *Ct* value is not determined (absent) in the channels for the FAM, ROX and Cy5 fluorophores. In such cases, the PCR analysis should be repeated.

The result of the analysis is considered reliable only if the results obtained for Positive and Negative Controls of amplification as well as for the Negative Control of extraction are correct (see Table 3 and 4).

Table 3

Results for controls

Control	Stage for control	*Ct* value in the channel for fluorophore	
		FAM, ROX	Cy5
C–	DNA extraction	Absent	< boundary value
NCA	PCR	Absent	Absent
C+	PCR	< boundary value	< boundary value

Table 4

Boundary *Ct* values

Sample	Rotor-type instrument			Plate-type instrument		
	Channel for fluorophore					
	FAM	ROX	Cy5	FAM	ROX	Cy5
C-	*Ct* is absent		33	*Ct* is absent		36
NCA	*Ct* is absent			*Ct* is absent		
C+	33	33	33	36	36	36
Test samples	-	-	33	-	-	36

10 TROUBLESHOOTING

Results of analysis are not taken into account in the following cases:

1. If the *Ct* value determined for the Positive Control of Amplification (C+) in the channels for the FAM and ROX fluorophores is greater than the boundary *Ct* value or absent, the amplification should be repeated for the samples for which *Ct* value is absent.
2. If the *Ct* value is determined for the Negative Control of Amplification (NCA) and/or Negative Control of Extraction (C–) in the channels for the FAM and/or ROX fluorophores, the PCR analysis starting from the DNA extraction stage should be repeated for all samples for which a *Ct* value was determined in the channels for the FAM and/or ROX fluorophores.

11 TRANSPORTATION

eSens N.gonorrhoeae /M.genitalium QL PCR kit should be transported at 2–8 °C for no longer than 5 days.

12 STABILITY AND STORAGE

All components of the **eSens N.gonorrhoeae /M.genitalium QL PCR kit** (except for polymerase (TaqF) and PCR-mix-2-FRT) are to be stored at the temperature 2–8 °C when not in use. All components of the **eSens N.gonorrhoeae /M.genitalium QL PCR kit** are stable until the expiry date stated on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

NOTE: Polymerase (TaqF) and PCR-mix-2-FRT are to be stored at the temperature from minus 24 to minus 16 °C when not in use.

NOTE: PCR-mix-1-FL N.gonorrhoeae/ M.genitalium is to be kept away from light.

13 SPECIFICATIONS

13.1 Sensitivity

Clinical material	**Nucleic acid extraction kit**	**PCR kit**	**Microorganism**	**Sensitivity, GE/ml***
Urogenital swabs	**DNA-sorb-AM** **ePure STD DNA Extraction Kit**	ES3049A	*Neisseria gonorrhoeae*	$5x10^2$
			Mycoplasma genitalium	10^3
Urine**	**DNA-sorb-AM** **ePure STD DNA Extraction Kit**	ES3049A	*Neisseria gonorrhoeae*	10^3
			Mycoplasma genitalium	$2x10^3$

*The quantity of genome equivalents of microorganism per 1 ml of the sample from transport medium.

** Pretreatment is required.

The analytical sensitivity for each microorganism is preserved in the presence of high DNA concentrations of other analyte microorganisms (up to 10^9 GE/ml).

13.2 Specificity

The analytical specificity of **eSens N.gonorrhoeae /M.genitalium QL PCR kit** is ensured by selection of specific primers and probes as well as stringent reaction conditions. The primers and probes were checked for possible homologies to all sequences published in gene banks by sequence comparison analysis.

Nonspecific responses were absent in tests of human DNA samples and DNA samples of the following microorganisms: *Gardnerella vaginalis, Lactobacillus spp.*, *Escherichia coli*, *Staphylococcus* spp., *Streptococcus* spp., *Candida albicans, Ureaplasma urealyticum, Ureaplasma parvum, Mycoplasma hominis, Mycoplasma genitalium, Neisseria* spp., *Neisseria gonorrhoeae, Trichomonas vaginalis, Treponema pallidum, Toxoplasma gondii, HSV* type 1 and 2, *CMV*, and *HPV*.

The clinical specificity of **eSens N.gonorrhoeae /M.genitalium QL PCR kit** was confirmed in laboratory clinical trials.

14 QUALITY CONTROL

The production process, including batch release, is carried out in accordance with an established quality management system certified according to ISO 13485.

15 KEY TO SYMBOLS USED

REF	Catalogue number
LOT	Batch code
IVD	*In vitro* diagnostic medical device
VER	Version
	Temperature limit
	Manufacturer
	Date of manufacture
EC REP	Authorized representative in the European Community

	Caution
	Contains sufficient for <n> tests
	Use-by Date
	Consult instructions for use
	Keep away from sunlight
NCA	Negative control of amplification
C-	Negative control of extraction
C+	Positive control of amplification
IC	Internal control

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
01_04/2022		

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