ECOLI

eSens

CE

IVD

For Professional Use Only

eSens Gardnerella vaginalis QL PCR kit

REF ES3005A

Instructions for Use

1 INTENDED USE

eSens Gardnerella vaginalis QL PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Gardnerella vaginalis* DNA in the clinical material (vaginal swabs) using real-time hybridization-fluorescence detection.

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

2 PRINCIPLE OF PCR DETECTION

Gardnerella vaginalis DNA detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific *Gardnerella vaginalis* primers. In the real-time PCR, the amplified product is detected with the use fluorescent dyes. These dyes are linked to oligonucleotide probes, which bind specifically to the amplified product during thermocycling. The real-time monitoring of fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run.

eSens Gardnerella vaginalis QL PCR kit is a qualitative test that contains the Internal Control (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 Gardnerella vaginalis QL PCR kit uses “hot-start,” which greatly reduces the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by the separation of nucleotides and Taq-polymerase by using chemically modified polymerase (TaqF). The 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.

Table 1

Channel for fluorophore	**FAM**	**JOE**
DNA-target	*Gardnerella vaginalis* DNA	Internal Control-FL (IC) DNA
Target gene	*gene 16S rRNA*	Artificially synthesized sequence

3 CONTENT

eSens Gardnerella vaginalis QL PCR kit (ES3005A) contains:

Reagent	**Description**	**Volume, ml**	**Quantity**
PCR-mix-1-FL *Gardnerella vaginalis*	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 Gardnerella vaginalis 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 barriers (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with a rotor for 2 ml 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 thin-walled PCR tubes with domed caps if a plate-type instrument is used;

- 0.2-ml thin-walled PCR tubes with flat caps or strips of four 0.1-ml Rotor-Gene PCR tubes if a rotor-type instrument is used).
- Refrigerator at the temperature from 2 to 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 filters 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 inhalation of vapors, samples and reagents contact with the skin, eyes, and mucous membranes. Harmful if swallowed. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice if necessary.
- 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.

NOTE: Some components of this kit contain sodium azide as a preservative. Do not use metal tubing for reagent transfer.

6 SAMPLING AND HANDLING

eSens Gardnerella vaginalis QL PCR kit is intended for the analysis of DNA extracted with DNA extraction kits from the clinical material (vaginal swabs).

7 WORKING CONDITIONS

eSens Gardnerella vaginalis 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 for each sample is carried out in the presence of **Internal Control-FL (IC).**

Please carry out nucleic acid extraction according to the manufacture´s instruction.

8.2 Preparing PCR

8.2.1 Preparing tubes for PCR

The type of tubes depends on the type of PCR real-time instrument.

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

1. Thaw the tube with **PCR-mix-2-FRT**. Vortex the tubes with **PCR-mix-1-FL *Gardnerella vaginalis***, **PCR-mix-2-FRT** and **Polymerase (TaqF)** and sediment the drops by short centrifugation (1–2 s).
2. Prepare the required number of tubes or strips for amplification of DNA from clinical and control samples
3. For carrying out N reactions (including 2 controls), mix in a new tube: **10*(N+1) µl of PCR-mix-1-FL *Gardnerella vaginalis*, 5.0*(N+1) µl of PCR-mix-2-FRT** and **0.5*(N+1) µl of Polymerase (TaqF)**.
4. Vortex the tube, then centrifuge it briefly.
5. Transfer **15 µl** of the prepared mixture into each tube.
6. Add **10 µl** of **DNA samples** obtained from clinical or control samples at the DNA extraction stage.
7. 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 (e.g Rotor-Gene Q or equivalent)			Plate-type Instruments (e.g CFX 96 Touch, CFX 96 Opus, QuantStudio 5 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 *fluorescent signal detection*		60	30 s *fluorescent signal detection*	
	72	15 s		72	15 s	

Fluorescent signal is detected in the channels for the FAM and JOE 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%
JOE/Yellow	from 4 FI to 8 FI	0.1	On	Off	5%

Test settings for plate-type instruments

Set the heating/cooling ***Ramp Rate* 2,5 °C/s**.

Channel	Threshold
FAM, JOE/HEX	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 two channels:

- The signal of the *Gardnerella vaginalis* DNA amplification product is detected in the channel for the FAM fluorophore.
- The signal of the Internal Control DNA amplification product is detected in the channel for the JOE 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 DNA sample in the corresponding column of the results grid.

Principle of interpretation is following:

- *Gardnerella vaginalis* DNA is **detected** in a sample if the *Ct* value determined in the results grid in the channel for the FAM fluorophore does not exceed the *Ct* value obtained for the Positive Control of Amplification (C+) or exceed it by not more than 2 cycles. Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.
- *Gardnerella vaginalis* DNA is **not detected** if the *Ct* value is not determined (absent) in the results grid (the fluorescence curve does not cross the threshold line) in the channel for the FAM fluorophore and the *Ct* value in the results grid in the channel for the JOE fluorophore does not exceed the specified boundary value
- The analysis result is **invalid** if the *Ct* value is not determined (absent) in the results grid in the channel for the FAM fluorophore and the Ct value in the results grid in the channel for the JOE fluorophore is not determined (absent) or exceeds the specified boundary value. In such cases PCR should be repeated.

The result of the analysis is considered reliable only if the results obtained for both 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	**JOE**
C–	DNA extraction	Absent	<boundary value
NCA	PCR	Absent	Absent
C+	PCR	<boundary value	<boundary value

Table 4

Boundary Ct values

	Rotor-type instrument		Plate-type instrument	
Sample	**Channel for fluorophore**			
	FAM	**JOE**	**FAM**	**JOE**
C+	33	30	36	33
NCA	Ct is absent		Ct is absent	
C-	Ct is absent	30	Ct is absent	33
Test samples	-	30	-	33

10 TROUBLESHOOTING

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

1. If the *Ct* value for the Positive Control of Amplification (C+) in the channel for the FAM fluorophore is greater than the boundary *Ct* value or absent, the amplification should be repeated for all samples in which *Gardnerella vaginalis* DNA was not detected.
2. If the *Ct* value is determined for the Negative Control of Extraction (C–) and/or Negative Control of Amplification (NCA) in the channel for the FAM fluorophore, the PCR analysis should be repeated from the DNA extraction stage for all samples in which *Gardnerella vaginalis* DNA was detected.

11 TRANSPORTATION

eSens Gardnerella vaginalis 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 Gardnerella vaginalis QL 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 **eSens Gardnerella vaginalis QL PCR kit** are stable until the expiration date on the label. PCR kit can be stored without unpacking at 2 to 8 °C for 3 months from the date of manufacture before opening. Once opened, PCR kit should be unpacked in accordance with the storage temperatures for each component. The shelf life of opened reagents is the same as that of unopened reagents, unless otherwise stated.

NOTE: Polymerase (TaqF) and PCR-mix-2-FRT are to be stored at temperature from minus 24 to minus 16 °C.

NOTE: PCR-mix-1-FL Gardnerella vaginalis should be kept away from light.

13 SPECIFICATIONS

13.1 Sensitivity

The analytical sensitivity of **eSens Gardnerella vaginalis QL PCR kit** is as follows:

Clinical material	Nucleic acid extraction kit	Sensitivity, GE/ml*
Urogenital swabs**	DNA-sorb-AM ePure STD DNA Extraction Kit	$1x10^4$

* Genome equivalents (GE) of the microorganism per 1 ml of a clinical sample placed in the transport medium specified.

** Urogenital swabs are to be placed into the **Transport Medium with Mucolytic Agent**.

13.2 Specificity

The analytical specificity of **eSens Gardnerella vaginalis 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 deposited in gene banks by sequence comparison analysis. Nonspecific reactions were absent while testing human DNA samples and a DNA panel of the following microorganisms: *Lactobacillus spp.; Escherichia coli; Staphylococcus* spp.; *Streptococcus* spp.; *Mycoplasma hominis; Ureaplasma urealyticum; Ureaplasma parvum; Candida albicans; Neisseria* spp.; *Neisseria gonorrhoeae; Mycoplasma genitalium; Trichomonas vaginalis; Treponema pallidum; Toxoplasma gondii; HSV* of 1 and 2 types, *CMV* and *HPV*.

The clinical specificity of **eSens Gardnerella vaginalis 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	⚠	Caution
LOT	Batch code	Σ	Contains sufficient for <n> tests
IVD	*In vitro diagnostic* medical device	⌛	Use-by Date
VER	Version		Consult instructions for use
	Temperature limit		Keep away from sunlight
	Manufacturer	NCA	Negative control of amplification
	Date of manufacture	C-	Negative control of extraction
EC REP	Authorized representative in the European Community	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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