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For Professional Use Only

eSens Gardnerella vaginalis/Lactobacillus sp. QT PCR kit

REF ES3043A

Instructions for Use

1 INTENDED USE

eSens Gardnerella vaginalis/Lactobacillus sp. QT PCR kit is an *in vitro* nucleic acid amplification test for qualitative and quantitative detection of *Gardnerella vaginalis* and *Lactobacillus* species DNA in the biological material (discharge of posterior fornix of vagina) 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

Gardnerella vaginalis and *Lactobacillus* species detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using special *Gardnerella vaginalis* and *Lactobacillus* species primers. In real-time PCR the amplified product is detected using 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 the reaction tubes after the PCR run.

eSens Gardnerella vaginalis/Lactobacillus sp. QT 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 chemically modified polymerase (TaqF). Chemically modified polymerase (TaqF) is activated by heating at 95°C for 15 min.

3 CONTENT

eSens Gardnerella vaginalis/Lactobacillus sp. QT PCR kit (ES3043A) includes:

Reagent		Description	Volume, ml	Quantity
PCR-mix-1-FRT *Gardnerella vaginalis / Lactobacillus* spp.		clear liquid from colorless to light lilac colour	0.8	1 tube
PCR-buffer-FRT		colorless clear liquid	0.9	1 tube
Polymerase (TaqF)		colorless clear liquid	0.06	1 tube
DNA-buffer		colorless clear liquid	0.5	1 tube
DNA calibrators PC *Gardnerella vaginalis / Lactobacillus* spp.	**GL1**	colorless clear liquid	0.06	1 tube
	GL2	colorless clear liquid	0.06	1 tube
	GL3	colorless clear liquid	0.06	1 tube
Positive Control DNA *Gardnerella vaginalis / Lactobacillus* spp.-1	**BV-**	colorless clear liquid	0.05	1 tube
Positive Control DNA *Gardnerella vaginalis / Lactobacillus* spp.-2	**BV+**	colorless clear liquid	0.05	1 tube

eSens Gardnerella vaginalis/Lactobacillus sp. QT 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 filter tips (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 2 ml reaction tubes.
- PCR box.
- Personal thermocyclers (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-typeinstrument is used;
- 0.2-ml PCR tubes with flat caps or strips of four 0.1-ml Rotor-Gene PCR tubes if arotor-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 barriers and use a new tip for every procedure.
- Store all extracted positive material (specimens, controls and amplicons) away from allother 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 samplesand reagents handling. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory workareas.
- 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 Gardnerella vaginalis/Lactobacillus sp. QT PCR kit is intended for analysis of the DNA extracted with DNA extraction kits from the biological material (discharge of posterior fornix of vagina).

7 WORKING CONDITIONS

eSens Gardnerella vaginalis/Lactobacillus sp. QT PCR kit should be used at 18–25 °C.

8 PROTOCOL

8.1 DNA extraction

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)**

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

C–	Add **100 µl** of **Transport Medium with Mucolytic Agent** to the tube labeled C–(Negative Control of Extraction) during extraction procedure.
C+	To the Positive Control of extraction tubes ((BV-) and (BV+)) transfer **90 µl** of**Transport Medium with Mucolytic Agent** (per each) and **10 µl** of **Positive Control DNA *Gardnerella vaginalis* / *Lactobacillus* spp.-1** or **Positive Control DNA *Gardnerella vaginalis* / *Lactobacillus* spp.-2** (respectively).

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.

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

1. Prepare the mixture of PCR-buffer-FRT and Polymerase (TaqF). Into the tube with PCR-buffer-FRT (0.9 ml) add all content of the tube of Polymerase (TaqF) (0.06 ml)and vortex carefully; avoid foaming. Label the tube indicating the date of preparation. Use disposable filter tips only.

   NOTE The prepared mixture is intended for 120 samples.

   The mixture can be stored at 2 – 8 °C for 3 months and used as necessary.

2. Prepare the required number of the tubes for amplification of DNA from clinical andcontrol samples.
3. Add reagents into the tubes (see Table 1).

Table 1

Methods of reagents addition

Method 1	Method 2
1. Add 7 µl of **PCR-mix-1-FRT *Gardnerella vaginalis* / *Lactobacillus* spp.** into each tube 2. Add above **8 µl of prepared mixture of PCR-buffer-FRT and Polymerase (TaqF)**	1. Prepare the reaction mixture for required number of reactions, calculating per each reaction: - **7 µl of PCR-mix-1-FRT *Gardnerella vaginalis* / *Lactobacillus* spp.** - **8 µl of prepared mixture of PCR-buffer-FRT and Polymerase (TaqF).** While calculating, take into account four controls (Negative Control and three Calibrators) and one extra reaction (see Table 2). 2. Add 15 µl of prepared mixture into the tubes.

Table 2

Scheme of reaction mixture preparation, in µl

Samples to be examined:	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
PCR-mix-1-FRT *Gardnerella vaginalis / Lactobacillus* spp., µl	56	63	70	77	84	91	98	105	112	119	126	133	140	147	154
Mixture of PCR-buffer-FRT and Polymerase (TaqF), µl	64	72	80	88	96	104	112	120	128	136	144	152	160	168	176
Samples to be examined:	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**
PCR-mix-1-FRT *Gardnerella vaginalis / Lactobacillus* spp., µl	161	168	175	182	189	196	203	210	217	224	231	238	245	252	259
Mixture of PCR-buffer-FRT and Polymerase (TaqF), µl	184	192	200	208	216	224	232	240	248	256	264	272	280	288	296

4. Using filter tips add **10 µl** of **DNA samples** obtained from clinical or control samples at the stage of DNA extraction into prepared tubes.

   NOTE Avoid transferring of sorbent into reaction mixture when adding DNA.

5. Carry out the control and calibration amplification reactions:

NCA	Add **10 µl** of **DNA-buffer** to the tube labeled NCA (Negative Control of Amplification) instead of the DNA-sample
Calibrators PC (GL1, GL2, GL3)	Into three tubes add **10 µl of each DNA-calibrator** (GL1, GL2, GL3)

8.2.2 Amplification

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

Table 3

Rotor-Gene 3000/6000 amplification program

Step	**Temperature °C**	**Time**	**Fluorescence detection**	**Cycles**
Hold	95	15 min	—	1
Cycling	95	10 sec	—	45
	60	40 sec	FAM/Green, JOE/Yellow	

NOTE Universal program**, eSens-1**, can be used as well (see table 5). The program allows conducting any combination of tests (for example, for detection of DNA of sexually transmitted infections) in a single run.

Analytical performances of the reagents kits remain the same when applying universal amplification program.

Table 4

Amplification program for plate-type instruments

Step	Temperature °C	Time	Fluorescence detection	Cycles
Hold	95	15 min	—	1
Cycling	95	20 sec	—	45
	60	1 min	FAM, JOE/HEX	

NOTE Universal program**, eSens-1**, can be used as well (see table 5). The program allows conducting any combination of tests (for example, for detection of DNA of sexually transmitted infections) in a single run.

Analytical performances of the reagents kits remain the same when applying universal amplification program.

Table 5

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 sec	5	95	5 sec	5
	60	20 sec		60	20 sec	
	72	15 sec		72	15 sec	
3	95	5 sec	40	95	5 sec	40
	60	20 sec fluorescent signal detection		60	30 sec fluorescent signal detection	
	72	15 sec		72	15 sec	

Fluorescent signal is detected in the channels for the FAM and JOE fluorophores. Other channels are enabled if several tests are simultaneously carried out in a single run.

2. Adjust the fluorescence channel sensitivity according to the Technical Sheet.
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.

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 ***Lactobacillus*** **spp. 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.

The quantity of copies per reaction for *Gardnerella vaginalis* DNA and *Lactobacillus* spp. DNA are calculated automatically by the software of the instrument using the specified calibrators values. The quantity of copies of *Gardnerella vaginalis* DNA and *Lactobacillus* spp. DNA are given in the corresponding column of the result grid.

Using the *Ct* values and specified values of calibrators GL1, GL2, GL3 a calibration curve plotting and calculation of *Gardnerella vaginalis* DNA and *Lactobacillus* spp. DNA copies per ml of initial clinical sample is performed automatically.

NOTE The *Gardnerella vaginalis* DNA and *Lactobacillus* spp. DNA concentrations for BV- and BV+ control samples are to be in the range specified in the *Technical Sheet* enclosed in the PCR kit.

Quantitative calibrators are used for quantitative detection of *Gardnerella vaginalis* and *Lactobacillus* spp. DNA copies in standard volume of clinical sample.

Calculation of concentrations of *Gardnerella vaginalis* and *Lactobacillus* spp. DNA per 1 ml of a biological material (posterior fornix of vagina discharge):

$$K_{DNA\ Gv/ml} = K_{DNA\ Gv} * \text{coefficient}$$

$$K_{DNA\ Lsp/ml} = K_{DNA\ Lsp} * \text{coefficient}$$

$K_{DNA\ Gv}$ = copies of *Gardnerella vaginalis* DNA per reaction

$K_{DNA\ Lsp}$ = copies of *Lactobacillus* spp. DNA per reaction,

Coefficient = 100 takes into account the volume of DNA in the reaction tube from the volume of the biological material and the quantity of copies of the amplified gene in the genome of the microorganism.

Calculation of relation coefficient of *Lactobacillus* spp. DNA and *Gardnerella vaginalis* DNA concentrations:

$$KC_{Lsp\text{-}Gv} = lg[K_{DNA\ Lsp/ml}] - lg[K_{DNA\ Gv/ml}]$$

KC < -1.0 – high possibility of BV

KC > 2.0 – low possibility of BV

10 TROUBLESHOOTING

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

1. If any *Ct* value appears for Negative Control of extraction and Negative Control of amplification in the channel for the FAM fluorophore (*Gardnerella vaginalis*) in the resultgrid it indicates contamination of the 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 to take measures to detect and eliminate the source of contamination.
2. If ***Calc Conc*** >50 appears for Negative Control of extraction and/or if ***Calc Conc*** >5 appears for Negative Control of amplification in the channel for the JOE fluorophore (*Lactobacillus* spp.) in the result grid it indicates contamination of the 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 to take measures to detect and eliminate the source of contamination.
3. If concentration values of *Gardnerella vaginalis* and *Lactobacillus* spp. DNA for control samples ((BV-) and (BV+)) do not fall in a range specified in the *Technical Sheet* it indicates the errors made during extraction or amplification stages.In this case it is necessary to repeat the PCR analysis.

11 TRANSPORTATION

eSens Gardnerella vaginalis/Lactobacillus sp. QT 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/Lactobacillus sp. QT PCR kit** (except for Polymerase (TaqF) and PCR-mix-1-FRT *Gardnerella vaginalis / Lactobacillus* spp.) are to be stored at 2–8 °C when not in use. All components of the **eSens Gardnerella vaginalis/Lactobacillus sp. QT 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-1-FRT** *Gardnerella vaginalis / Lactobacillus* spp. are to be stored at temperature from **- 24 to - 16 °C** when not in use.

Polymerase (TaqF) and **PCR-mix-1-FRT** *Gardnerella vaginalis / Lactobacillus* spp. are to be stored at temperature from **- 24 to - 16 °C** when not in use

13 SPECIFICATIONS

13.1 Analytical sensitivity

Biological material	Transport medium	Nucleic acid extraction kit	Microorganism	Sensitivity, copies/ml*
Discharge of posterior fornix of vagina	Transport Medium with Mucolytic Agent	DNA-sorb-AM ePure STD DNA Extraction Kit (E2007)	*Gardnerella vaginalis*	5×10^3
			Lactobacillus spp	5×10^3

* The quantity of the pathogen agent DNA copies per 1 ml of a clinical sample, placed into the specified transport medium.

13.2 Analytical specificity

The analytical specificity of **eSens Gardnerella vaginalis/Lactobacillus sp. QT PCR kit** is ensured by selection of specific primers and probes, as well as the selection of stringent reaction conditions. The primers and probes were checked for possible homologies to all in gene banks published sequences by sequence comparison analysis.

Nonspecific responses were absent in tests of human DNA samples and the panel of the following microorganisms DNA samples: *Staphylococcus* spp., *Streptococcus* spp., *Candida albicans, Candida glabrata, Candida krusei, Mycoplasma hominis, Ureaplasma urealyticum, Ureaplasma parvum, Mycoplasma genitalium, Chlamydia trachomatis,Neisseria* spp., *Neisseria gonorrhoeae, Trichomonas vaginalis, Treponema pallidum, Toxoplasma gondii, HSV types* 1 and 2, *CMV, HPV* and human DNA.

13.3 Linear measurement range

The linear measurement range for quantitative estimation of each detected microorganism is from 10^3 to 10^7 copies/ml.

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	BV- BV+	Positive control of extraction

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
01_04/2022		

Ecoli Dx, s.r.o. , Purkyňova 74/2

110 00 Praha 1, Česká republika
Tel: +420 325 209 912

Mobil: +420 739 802 523