



For *in Vitro* Diagnostic Use

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AmpliSens® *Gardnerella vaginalis*-FRT

PCR kit

Instruction Manual

AmpliSens®



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1. INTENDED USE.

AmpliSens® Gardnerella vaginalis-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Gardnerella vaginalis* DNA in the clinical materials (scrapes (swabs) of urogenital tract mucous membranes; urine sediment) by means of real-time hybridization-fluorescence detection.

2. PRINCIPLE OF PCR DETECTION.

Gardnerella vaginalis detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using special *Gardnerella vaginalis* primers. In real-time PCR the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes which bind specifically to the amplified product. 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. **AmpliSens® Gardnerella vaginalis-FRT** PCR kit is a qualitative test and contains the Internal Control (IC) which must be used in the isolation procedure in order to control the isolation process of each individual sample and to identify possible reaction inhibition. **AmpliSens® Gardnerella vaginalis-FRT** 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). The wax melting and reaction components mixing occur only at 95 °C. Chemically modified polymerase (TaqF) activates by heating at 95 °C for 15 min.

3. CONTENT.

AmpliSens® Gardnerella vaginalis-FRT PCR kit is produced in 3 forms:

AmpliSens® *Gardnerella vaginalis*-FRT PCR kit variant FRT (for use with RG) **REF** R-B7(RG)-CE.

AmpliSens® *Gardnerella vaginalis*-FRT PCR kit variant FRT (for use with iQ) **REF** R-B7(iQ)-CE.

AmpliSens® *Gardnerella vaginalis*-FRT PCR kit variant FRT-100 F (for use with RG, iQ) **REF** R-B7-F(RG,iQ)-CE.

AmpliSens® Gardnerella vaginalis-FRT PCR kit, variant FRT includes:

Reagent	Description	Volume (ml)	Amount
PCR-mix-1-FEP/FRT Gardnerella vaginalis (under wax)	colorless, clear liquid	0.008	110 tubes of 0.2 ml
PCR-mix-2-FL	colorless, clear liquid	0.77	1 tube
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**	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, protocol).

AmpliSens® *Gardnerella vaginalis*-FRT PCR kit is intended for 110 reactions, including controls.

AmpliSens® Gardnerella vaginalis-FRT PCR kit, variant FRT-100 F includes:

Reagent	Description	Volume (ml)	Amount
PCR-mix-1-FEP/FRT Gardnerella vaginalis	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 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**	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, protocol).

AmpliSens® *Gardnerella vaginalis*-FRT PCR kit is intended for 110 reactions, including controls.

4. ADDITIONAL REQUIREMENTS.

- DNA isolation kit.
- Disposable powder-free gloves.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol filters 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 (0.2) ml capacity (for example, “Axygen”, USA).
- Refrigerator for 2–8 °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 filters 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 unidirectional 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 material samples for PCR-analysis, transportation and storage are described in manufacturer's handbook [1]. It is recommended that this handbook is read before starting of the work.

AmpliSens® *Gardnerella vaginalis*-FRT PCR kit is intended for the analysis of DNA extracted with DNA isolation kits from

- scrapes (swabs) of urogenital tract mucous membranes;
- urine sediment (use the first portion of the morning urine specimen).

7. PROTOCOL.

7.1. DNA Isolation

It's recommended to use the following nucleic acid extraction kits:

- "DNA-sorb-AM", **REF** K1-12-100-CE.



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 *Gardnerella vaginalis*** 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 *Gardnerella vaginalis***.

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 *Gardnerella vaginalis***, **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 *Gardnerella vaginalis* 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 complex** 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, ROX/Orange, Cy5/Red	
	72	15 sec	—	

Fluorescence detection (Acquiring to Cycling A) is on the 2-nd pass (**60°C**) in FAM/Green,

JOE/Yellow, ROX/Orange and Cy5/Red fluorometer channels (ROX/Orange and Cy5/Red channels are activated as necessary if they are used in an applied “multiprime” format test).



AmpliSens-1 RG general program allows simultaneous conducting of any combination of tests for detection of pathogens of sexually transmitted diseases 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 (by means of AmpliSens PCR kits) are carried out simultaneously, the program and template corresponding to those “multiprime” tests should be applied.

3. Make the adjustment of the fluorescence channel sensitivity according to Appendix 1.

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, HEX	
	72	15 sec	–	

Fluorescence detection is on the 2-nd pass (**60°C**) in FAM, HEX fluorometer channels.



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

3. Make the adjustment of the fluorescence channel sensitivity according to Appendix 2.

8. DATA ANALYSIS.

RG. Internal Control is detected in the JOE /Yellow fluorescence channel, *Gardnerella vaginalis* DNA is detected in 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 in the HEX fluorescence channel, *Gardnerella vaginalis* DNA is detected in the FAM fluorescence channel.

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

Results interpretation

The results are interpreted by the software of used device by the crossing (or not) of fluorescence curve with the threshold line.

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 (< Y*)	Neg	OK

*For X, Y values see Appendices 1, 2.

1. The sample is considered positive for *Gardnerella vaginalis* if its Ct value is defined in the results grid in FAM/Green channel.
2. The sample is considered negative for *Gardnerella vaginalis* if its Ct value is not defined in the results grid (the fluorescence curve does not cross the threshold line) in FAM/Green channel while in the results grid in the JOE/Yellow/HEX channel the Ct value doesn’t exceed X.
Results are accepted as relevant if both positive and negative controls of amplification along with negative control of extraction are passed.

9. TROUBLESHOOTING.

Results of analysis are not being registered in the following cases:


1. If Ct value is absent in both JOE/Yellow/HEX and FAM/Green channels or the Ct value in JOE/Yellow/HEX channel is higher than X, PCR reaction should be repeated. If the same result is obtained, the sample analysis should be repeated starting from the extraction stage.
2. If the signal is registered in Negative Control of extraction (C-) in FAM/Green channel and/or in Negative Control of amplification (NCA) in 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 necessary to repeat the analysis, and also to take measures to detect and eliminate the source of contamination.
3. If no signal is detected for Positive Control of amplification, it can suggest of incorrect programming of the temperature profile of the 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. Programming of Instrument, storage conditions, and the expiration date of the reagents should be checked, and then the PCR should be repeated.
4. If a positive result (fluorescence curve crosses the threshold line) is registered for the sample that has a fluorescence curve without a typical exponential growth (the graph is linear). This can suggest of incorrect setting of the threshold line or incorrect calculation of base line parameters. Such a result should not be considered as positive. Once the threshold line has been set correctly, the PCR should be repeated for the sample. (This option is applicable only

for iQ instruments).

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

10. STABILITY AND STORAGE.


All components of the **AmpliSens® Gardnerella vaginalis-FRT** PCR kit (except for polymerase (TaqF) and PCR-mix-2-FRT) are to be stored between 2°C and 8°C, when not in use. All components of the PCR kit must be stable until the expiry date stated on the label.

 Polymerase (TaqF) and PCR-mix-2-FRT are to be stored at not more than minus 16 °C. PCR-mix-1-FEP/FRT *Gardnerella vaginalis* is to be stored in a light-protected place.

11. SPECIFICATIONS.

11.1. Sensitivity.

Analytical Sensitivity of **AmpliSens® Gardnerella vaginalis-FRT** PCR kit is no less than 1x10³ genome equivalents per 1 ml of sample (GE/ml).

 The claimed analytical features of **AmpliSens® Gardnerella vaginalis-FRT** PCR kit are guaranteed only when additional reagents kit, “DNA-sorb-AM” (manufactured by Federal State Institution of Science Central Research Institute of Epidemiology), is used.

11.2. Specificity.

Specificity of **AmpliSens® Gardnerella vaginalis-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® Gardnerella vaginalis-FRT** PCR kit was confirmed in laboratory clinical trials.










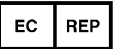






12. REFERENCES.

1. 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® Gardnerella vaginalis-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
	Contains sufficient for <n> tests		Authorised representative in the European Community.
	Consult instructions for use		Caution, consult accompanying documents
	For working with Rotor-Gene™ 3000/6000 (Corbett Research)		For working with iQ5, iQiCycler (Bio-Rad)
	Positive Control		Negative Control