



For Professional Use Only

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 clinical materials (urogenital swabs) by using real-time hybridization-fluorescence detection.



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 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 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. **AmpliSens® *Gardnerella vaginalis*-FRT** PCR kit is a qualitative test that contains the Internal Control (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. **AmpliSens® *Gardnerella vaginalis*-FRT** 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 chemically modified polymerase (TaqF). Wax melts and reaction components mix only at 95 °C.

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)	Quantity
PCR-mix-1-FL <i>Gardnerella vaginalis</i> (ready-to-use single-dose test tubes (<i>under wax</i>))	colorless clear liquid	0.01	110 tubes of 0.2 ml
PCR-mix-2-FL-red	red clear liquid	1.1	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 (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 during the DNA extraction procedure directly to the sample/lysis mixture (see DNA-sorb-AM **REF** K1-12-100-CE, DNA-sorb-B, **REF** K1-2-100-CE protocols).

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)	Quantity
PCR-mix-1-FL <i>Gardnerella vaginalis</i>	colorless clear liquid	1.2	1 tube
PCR-mix-2-FRT	colorless clear liquid	0.3	2 tubes
Polymerase (TaqF)	colorless clear liquid	0.03	2 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 (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 during the DNA extraction procedure directly to the sample/lysis mixture (see DNA-sorb-AM **REF** K1-12-100-CE, DNA-sorb-B, **REF** K1-2-100-CE protocols).

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

4. ADDITIONAL REQUIREMENTS

- DNA extraction kit
- Transport medium.
- 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.
- Personal thermocyclers (for example, Rotor-Gene 3000 or Rotor-Gene 6000 (Corbett Research, Australia); Rotor-Gene Q (Qiagen, Germany), iCycler iQ or iQ5 (Bio-Rad, USA), Mx3000P (Stratagene, USA) or equivalent).
- Disposable polypropylene microtubes for PCR (0.2- or 0.1-ml; for example, Axygen, USA).
- Refrigerator for 2–8 °C.
- Deep-freezer for ≤ –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 and handle amplicons away from all other reagents.
- Thaw all components thoroughly at room temperature before starting detection.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats, protect eyes while samples and reagents handling. Thoroughly wash hands afterward.
- 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 samples and unused reagents in compliance with local authorities' requirements.
- Samples 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 another suitable disinfectant.

- Avoid contact with the skin, eyes, and mucosa. If skin, eyes, or mucosa contact, immediately flush with water, seek medical attention.
- 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.
- The laboratory process must be one-directional, it should begin in the Extraction Area and then move 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 samples of biological materials for PCR-analysis, transportation and storage are described in the manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

AmpliSens® *Gardnerella vaginalis*-FRT PCR kit is intended for the analysis of DNA extracted by DNA extraction kits from urogenital swabs.

7. WORKING CONDITIONS

AmpliSens® *Gardnerella vaginalis*-FRT PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. DNA extraction

It is recommended to use the following nucleic acid extraction kits:

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



Extract DNA according to the manufacturer's instructions.

8.2. Preparing PCR

8.2.1. Preparing tubes for PCR

Variant FRT

The total reaction volume is **30 µl**, the volume of DNA sample is **10 µl**.

1. Prepare the required number of tubes with **PCR-mix-1-FL *Gardnerella vaginalis*** and wax for amplification of DNA from clinical and control samples.
2. Add **10 µl** of **PCR-mix-2-FL-red** to the surface of the wax layer of each tube ensuring that it does not fall under the wax and mix with **PCR-mix-1-FL *Gardnerella vaginalis***.
3. Add **10 µl** of **DNA** obtained from clinical or control samples at the DNA extraction stage

into the prepared tubes using tips with aerosol barrier.

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 sample isolated from **Negative Control** to the tube labeled C- (Negative Control of Extraction).

Variant FRT-100 F

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

1. Thaw the tube with **PCR-mix-2-FRT**. Prepare the required quantity of tubes with **PCR-mix-1-FL *Gardnerella vaginalis***, **PCR-mix-2-FRT** and **Polymerase (TaqF)** and sediment the drops by short centrifuging (1–2 s).

2. Prepare the required quantity 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. Mix and precipitate the drops by short centrifuging.

5. Transfer **15 µl** of the prepared mixture into each tube.

6. Add **10 µl** of **DNA** obtained from clinical or control samples at the DNA extraction stage into the prepared tubes using tips with aerosol barrier.

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 sample isolated from **Negative Control** to the tube labeled C- (Negative Control of Extraction).

8.2.2. Amplification

Program the real-time amplification instrument according to manufacturer's manual and Guidelines [2].

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

AmpliSens-1 program

Step	Rotor-type Instruments ¹			Plate-type Instruments ²		
	Temperature, °C	Time	Cycles	Temperature, °C	Time	Cycles
Hold	95	15 min	1	95	15 min	1
Cycling 1	95	5 s	5	95	5 s	5
	60	20 s		60	20 s	
	72	15 s		72	15 s	
Cycling 2	95	5 s	40	95	5 s	40
	60	20 s <i>fluorescent signal detection</i>		60	30 s <i>fluorescent signal detection</i>	
	72	15 s		72	15 s	

Fluorescent signal is detected in the channels designed for the FAM/Green and JOE/Yellow/HEX fluorophores (other channels are enabled if several tests are simultaneously carried out in a single run).

- Adjust the fluorescence channel sensitivity according to *Important Product Information Bulletin*.
- Insert tubes into the reaction module of the device.
- Run the amplification program with fluorescence detection.
- Analyze results after the amplification program is completed.

9. DATA ANALYSIS

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

The Internal Control DNA is detected in the JOE fluorescence channel, the *Gardnerella vaginalis* DNA is detected in the FAM fluorescence channel.

See the **Manufacturer's manual, Guidelines** and **Important product information bulletin** for data analysis settings.

Principle of interpretation:

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

- Gardnerella vaginalis* DNA is **detected** in a sample if its Ct value is defined in the results grid in the FAM channel and it is not greater than the threshold value obtained for Positive Control of Amplification (C+) or it is not greater than it more than 2 cycles.
- Gardnerella vaginalis* DNA is **not detected** if its Ct value is not defined in the results grid (the fluorescence curve does not cross the threshold line) in the FAM channel and the Ct

¹ For example, Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q or equivalent.

² For example, iCycler iQ5, Mx3000P, Mx3000 or equivalent.

value in the results grid in the JOE channel does not exceed the threshold value.

- The analysis result is **invalid** if the Ct value is not defined in the results grid (the fluorescence curve does not cross the threshold line) in FAM channel and the Ct value in the results grid in the JOE channel is not defined or exceeds the threshold value. In such cases PCR should be repeated.

The result of the analysis is considered to be reliable only if the results obtained for both Positive and Negative Controls of amplification as well as Negative Control of extraction are correct (see table 2).

Table 2

Results for controls

Control	Stage for control	Ct value in channel		Interpretation
		FAM	JOE	
C-	DNA isolation	Neg	Pos (<boundary value)*	OK
NCA	Amplification	Neg	Neg	OK
C+	Amplification	Pos (<boundary value)	Pos (<boundary value)	OK

*For boundary Ct values for samples, Negative Control of Extraction and Positive Control of Amplification, see the **Important Product Information Bulletin**.

10. TROUBLESHOOTING

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

1. If the Ct value is absent in both JOE/Yellow/HEX and FAM/Green channels or the Ct value in the JOE/Yellow /HEX channel is higher than the specified boundary value, PCR should be repeated. If the same result is obtained, the extraction stage for the sample should be repeated. If the IC signal of this sample was detected normally in any other PCR test, it is not necessary to repeat the extraction stage (for iCycler iQ or iQ5 instruments).
2. If the Ct value is present for C- in the FAM/Green channel and/or for NCA in the FAM, JOE/Yellow /HEX channels in the results grid, it indicates contamination of reagents or samples. In such cases, the results of analysis must be considered as invalid. Test analysis must be repeated and measures to detect and eliminate the source of contamination must be taken.
3. If no signal is detected for the Positive Controls of amplification, it may suggest that the programming of the temperature profile of the used Instrument was incorrect, or that the configuration of the PCR reaction was incorrect, or that the storage conditions for kit components has not complied with the manufacturer’s instruction, or that the reagent kit has expired. Programming of the used instrument, storage conditions, and the expiration

date of the reagents should be checked, and then PCR should be repeated.

4. If a positive result (the fluorescence curve crosses the threshold line) is detected for a sample that has a fluorescence curve without the typical exponential growth phase (the curve is linear), this may suggest incorrect setting of the threshold line or incorrect calculation of baseline parameters. Such a result should not be considered as positive. Once the threshold line has been set correctly, PCR analysis of the sample should be repeated (if iCycler iQ or iQ5 instruments are used).

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

11. TRANSPORTATION

AmpliSens® Gardnerella vaginalis-FRT PCR kit should be transported at 2–8 °C for no longer than 5 days.

12. 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 at 2–8 °C when not in use. All components of the **AmpliSens® Gardnerella vaginalis-FRT** PCR kit are stable until the expiration date on the label. The shelf life of opened reagents is the same as that of unopened reagents, unless otherwise stated.



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



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

13. SPECIFICATIONS

13.1. Sensitivity

Analytical Sensitivity of **AmpliSens® Gardnerella vaginalis-FRT** PCR kit is following:

Clinical material	Nucleic acid extraction kit	Sensitivity, GE/ml ³
Urogenital swabs ⁴	DNA-sorb-AM	1x10 ⁴

³ Genome equivalents (GE) of the microorganism per 1 ml of a clinical sample placed in the transport medium specified.

⁴ Urogenital swabs are to be placed into the Transport Medium for Swabs (**REF** 956-CE, 987-CE) or Transport Medium with Mucolytic (**REF** 952-CE, 953-CE).

13.2. Specificity

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














14. REFERENCES

1. Handbook “Sampling, Transportation, and Storage of Clinical Material for PCR Diagnostics”, developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow, 2008.
2. Guidelines “Real-Time PCR Detection of STIs and Other Reproductive Tract Infections”, developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow.

15. QUALITY CONTROL

In compliance with Federal Budget Institute of Science “Central Research Institute for 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.

16. KEY TO SYMBOLS USED

	Catalogue number		Caution
	Batch code		Sufficient for
	<i>In vitro</i> diagnostic medical device		Expiration Date
	Version		Consult instructions for use
	Temperature limitation		Keep away from sunlight
	Manufacturer	NCA	Negative control of amplification
	Date of manufacture	C-	Negative control of extraction
	Authorised representative in the European Community	C+	Positive control of amplification
FBIS CRIE	Federal Budget Institute of Science “Central Research Institute for Epidemiology”	IC	Internal control

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
19.06.11 RT	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”