



For *in Vitro* Diagnostic Use



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AmpliSens® HSV I, II-FRT PCR kit

Instruction Manual



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1. INTENDED USE

AmpliSens® HSV I, II-FEP PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Herpes Simplex virus* types I and II (*HSV1, II*) DNA in the biological material (scrapes (swabs) of urogenital tract mucous membranes; papules, vesicles, or ulcers fluid; urine sediment) by using end-point hybridization-fluorescence detection of amplified products.

2. PRINCIPLE OF PCR DETECTION

Herpes simplex virus types I, II detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using special *HSV I, II* primers. In Fluorescent End-Point PCR, the amplified product is detected by using fluorescent dyes. These dyes are usually linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. A multi channel rotor-type fluorometer is specially designed to detect fluorescent excitation from the fluorophores in a reaction mix after PCR. It allows the accumulating product detection without re-opening the reaction tubes after the PCR run. **AmpliSens® HSV I, II-FEP** PCR kit is a qualitative test, which contains the Internal Control (IC). It 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® HSV I, II-FEP** 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). Wax melting and reaction mix components occur only at 95°C. Chemically modified polymerase (TaqF) is activated by heating at 95°C for 15 min.

3. CONTENT

AmpliSens® HSV I, II-FRT PCR kit is produced in 3 forms:

AmpliSens® *HSV I, II-FRT* PCR kit variant FRT (for use with RG) **REF** R-V8(RG)-CE,

AmpliSens® *HSV I, II-FRT* PCR kit variant FRT (for use with iQ) **REF** R-V8(iQ)-CE,

AmpliSens® *HSV I, II-FRT* PCR kit variant FRT-100 F (for use with RG, iQ) **REF** R-V8-F(RG, iQ)-CE

AmpliSens® HSV I, II-FRT PCR kit, variant FRT includes:

Reagent	Description	Volume (ml)	Quantity
PCR-mix-1-FEP/FRT <i>HSV I, II (under wax)</i>	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 (IC)**	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® *HSV I, II-FRT* PCR kit is sufficient for 110 reactions, including controls.

AmpliSens® HSV I, II-FRT PCR kit, variant FRT-100 F includes:

Reagent	Description	Volume (ml)	Quantity
PCR-mix-1-FEP/FRT <i>HSV I, II</i>	colorless clear liquid	1.2	1 tube
PCR-mix-2-FRT	colorless clear liquid	0.3	2 tubes
Polymerase (TaqF)	colorless clear liquid	0.06	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 (IC)**	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® *HSV I, II-FRT* PCR kit is intended for 110 reactions, including controls.

4. ADDITIONAL REQUIREMENTS

- DNA isolation 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 rotor.
- PCR box.
- Personal thermocycler (for example, Rotor-Gene™ 3000 or Rotor-Gene™ 6000 (Corbett Research, Australia), iQ5 or iQ iCycler (BioRad, USA))
- Disposable polypropylene microtubes for PCR (for example, “Axygen”, USA).
- Refrigerator for temperature between 2 and 8 °C.
- Deep-freezer with temperature not more than minus16 °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 other suitable disinfectant.
- Avoid contact with the skin, eyes and mucosa. If skin, eyes and 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 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 is described in manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

AmpliSens® HSV I, II-FEP PCR kit is intended for analysis of DNA extracted by using DNA isolation kits from urogenital tract mucous membranes scrapes (swabs); fluid of papules, vesicles, ulcers; urine sediment (first portion of the morning 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.



Please carry out the DNA isolation according to the manufacturer instruction.

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. Prepare the required number of the tubes with **PCR-mix-1-FEP/FRT HSV I, II** 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, ensuring that it doesn't fall under the wax and mix with **PCR-mix-1-FEP/FRT HSV I, II**.

Variant FRT-100 F

1. Prepare required number of the tubes for amplification of DNA from 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: $10 \cdot (N+1)$ µl of **PCR-mix-1-FEP/FRT HSV I, II**, $5.0 \cdot (N+1)$ µl of **PCR-mix-2-FRT** and $0.5 \cdot (N+1)$ µl of **polymerase (TaqF)**. Vortex the tube, then centrifuge shortly. Transfer **15 µl** of prepared mix into each tube.
Steps 3 and 4 are effective 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 HSV I, II that are not used at the moment should be kept away from light.

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).

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	
	72	15 sec	–	



It is possible to use **AmpliSens-1 RG** general program that allows simultaneous conducting of any combination of tests for detection of sexually transmitted diseases pathogens DNA including tests for identifying of *Human Papillomaviruses* by using of AmpliSens HPV HCR PCR kits.

3. Fluorescence detection is on the 2-nd pass (**60°C**) in FAM/Green and JOE/Yellow fluorometer channels.
4. 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	–	



It is possible to use **AmpliSens-1 iQ** general program that allows simultaneous conducting of any combination of tests for detection of sexually transmitted diseases pathogens DNA including tests for identifying of *Human Papillomaviruses* by using of AmpliSens HPV HCR PCR kits.

3. Fluorescence detection is on the 2-nd pass (**60°C**) FAM and HEX in fluorometer channels.
4. 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, *HSV I*, II DNA is detected on 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, *HSV I*, II DNA is detected on the FAM fluorescence channel. See **Appendix 2** for data analysis settings for iQ5 or iQ iCycler.

Results interpretation

The results are interpreted with the software of Rotor-Gene™ 3000 or Rotor-Gene™ 6000 Instrument through the presence (or absence) of crossing of fluorescence curve with the threshold line.

Results for controls

Control	Stage for control	Ct channel FAM /Green	Ct channel JOE/Yellow/ HEX	Interpretation
C-	DNA isolation	Neg	Pos (< X*)	OK
NCA	Amplification	Neg	Neg	OK
C+	Amplification	Pos (< Z*)	Neg	OK

*For X, Z values see Appendix 1 in case of Rotor-Gene™ 3000 or Rotor-Gene™ 6000 Instrument is used or Appendix 2 in case of iQ5 or iQiCycler Instrument is used.

1. The sample is considered to be positive if its Ct value is defined in the results grid in FAM/Green channel.
2. The sample is considered to be negative if its Ct value is not defined in the results grid (the fluorescence curve does not cross the threshold line) in FAM/Green channel and in the results grid on 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 channel is higher than X, it is necessary to repeat PCR reaction once again. If repeatedly received result is the same, it is required to re-do the analysis of the sample from the very beginning of the extraction. If IC signal of this sample was detected normally during any other PCR test, therefore, for second examination the extraction stage should be omitted.
2. If the signal is registered in Negative Control of extraction (C-) on 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 of all tests, and also to take measures to detect and eliminate the source of contamination.
3. If no signal is detected for Positive Controls of amplification, it can suggest incorrect programming of the temperature profile, 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, storage conditions, and the expiration date of the reagents should be checked, and then the PCR should be repeated.

- If positive result (fluorescence curve crosses the threshold line) is registered for the sample that has fluorescence curve without typical exponential growth (the graph is linear). It can suggest of incorrect threshold line setting or incorrect calculation of base line parameters. Such a result should not be considered as positive. If the threshold line has been set correctly, the PCR should be repeated for the sample. (in case of iQ Instrument is used)

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[®] HSV I, II-FRT** PCR kit (except for Polymerase (TaqF) and PCR-mix-2-FRT) are to be stored at the temperature between 2 °C and 8 °C, when not in use. All components of the **AmpliSens[®] HSV I, II-FRT** PCR kit are to be stable until labeled expiration date.



Polymerase (TaqF) and PCR-mix-2-FRT are to be stored at the temperature not more than minus 16°C.

11. SPECIFICATIONS

11.1. Sensitivity.

Analytical Sensitivity of **AmpliSens[®] HSV I, II-FRT** PCR kit is no less than 1×10^3 genome equivalents per 1 ml of sample (GE/ml).



Claimed analytical features of **AmpliSens[®] HSV I, II-FEP** 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[®] HSV I, II-FRT** 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. Specificity of **AmpliSens[®] HSV I, II-FRT** PCR kit was confirmed in laboratory clinical trials.








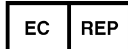








12. REFERENCES

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