



For in Vitro Diagnostic Use

AmpliSens[®] HSV-typing-FRT

PCR kit

Instruction Manual



Ecoli s.r.o., Studenohorska 12
841 03 Bratislava 47
Slovak Republic
Tel.: +421 2 6478 9336
Fax: +421 2 6478 9040

ecoli@ecoli.sk www.ecoli.sk
www.pcrdiagnostics.eu



Federal State Institution of Science
Central Research Institute of Epidemiology
3A Novogireevskaya Street
Moscow 111123 Russia



1. INTENDED USE.

AmpliSens[®] HSV-typing-FRT PCR kit is an in vitro nucleic acid amplification test for qualitative detection and typing of *Herpes Simplex virus* types I and II (HSV I and HSV II) DNA in the biological material (scrapes (swabs) of urogenital tract mucous membranes; papules, vesicles, or ulcers fluid; urine sediment) by using of real-time 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 the amplification of pathogen genome specific region using special HSV I, II 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. AmpliSens[®] HSV-typing-FRT PCR kit is a qualitative test, which contain 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-typing-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). 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-typing-FRT PCR kit is produced in 3 forms:

AmpliSens[®] HSV-typing-FRT PCR kit variant FRT (for use with RG) [REF](#) R-V38(RG)-CE.

AmpliSens[®] HSV-typing-FRT PCR kit variant FRT (for use with iQ) [REF](#) R-V38(iQ)-CE.

AmpliSens[®] HSV-typing -FRT PCR kit variant FRT-100 F (for use with RG, iQ) [REF](#) R-V38-F(RG, iQ)-CE

AmpliSens[®] HSV-typing-FRT PCR kit, variant FRT includes:

Reagent	Description	Volume (ml)	Quantity
PCR-mix-1-FEP/FRT HSV-typing ready-to-use single-dose test tubes (under wax)	colorless, clear liquid	0.008	110 tubes
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, "DNA-sorb-B" [REF](#) K1-2-100-CE protocols).

AmpliSens[®] HSV-typing-FRT PCR kit is intended for 110 reactions, including controls.

AmpliSens[®] HSV-typing-FRT PCR kit, variant FRT-100 F includes:

Reagent	Description	Volume (ml)	Quantity
PCR-mix-1-FEP/FRT HSV-typing	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, "DNA-sorb-B" [REF](#) K1-2-100-CE protocols).

AmpliSens[®] HSV-typing-FRT PCR kit is intended for 110 reactions, including controls.

4. ADDITIONALLY REQUIRED MATERIALS, REAGENTS AND DEVICES.

- 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 for 2 ml reaction tubes
- PCR box
- Rotor-Gene™ 3000 or Rotor-Gene™ 6000 (Corbett Research, Australia) Instrument; iQ5 or iQ1Cycler (BioRad, USA) Instrument
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity
- Refrigerator for 2–8 °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 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 detection.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats, 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 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-typing-FRT* PCR kit is intended for the analysis of DNA extracted with 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

Different manufacturers offer DNA isolation kits. We recommend following nucleic acid extraction kits:

- "DNA-sorb-AM", [REF](#) K1-11-100-CE.
- "DNA-sorb-B", [REF](#) K1-2-100-CE (if extracting DNA from whole blood or cerebrospinal fluid).



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.



If applying two-channel Instrument in which the channel for detection of Internal Control (ROX/Orange) is absent, it is necessary to confirm presence of Herpes simplex virus I, II DNA in clinical samples by using of AmpliSens® *HSV I, II-FRT* PCR kit.

Variant FRT

1. Prepare the required number of the tubes with **PCR-mix-1-FEP/FRT-typing** 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 does not fall under the wax and mix with **PCR-**

mix-1-FEP/FRT HSV-typing.

Variant FRT-100F

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 carrying of N reactions (including 2 controls) mix in a new tube: 10*(N+1) µl of **PCR-mix-1-FEP/FRT HSV-typing**, 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 DNA extraction stage into prepared tubes.



The tubes with PCR-mix-1-FEP/FRT *HSV* -typing 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, ROX/Orange	
	72	15 sec	–	



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

3. Fluorescence detection is on the 2-nd pass (60°C) in FAM/Green, JOE/Yellow and ROX/Orange 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, ROX	
	72	15 sec	–	



AmpliSens-1 iQ general program allows a combination of test for the detection of pathogens of sexually transmitted disease to be carried out simultaneously, including tests for the identification of *Human Papillomaviruses* using AmpliSens HPV HCR PCR kits.

- Fluorescence detection is on the 2-nd pass (**60°C**) in FAM, HEX and ROX fluorometer channels.
- Make the adjustment of the fluorescence channel sensitivity according to Appendix 2.

8. DATA ANALYSIS

RG. Internal Control is detected in the ROX/Orange fluorescence channel (for 4-channel instrument), *HSV I* DNA is detected in the JOE/Yellow fluorescence channel, *HSV II* DNA is detected in FAM/Green fluorescence channel. See **Appendix 1** for data analysis settings for applied instrument.

iQ. Internal Control is detected in the ROX fluorescence channel (for 4-channel instrument), *HSV I* DNA is detected in the HEX fluorescence channel, *HSV II* DNA is detected in FAM fluorescence channel. See **Appendix 2** for data analysis settings for applied instrument.

Results interpretation

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

Results for controls

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

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

Two-channel instrument.

- The sample is considered to be positive for HSV type II if its Ct value is defined in the results grid in FAM/Green channel.
- The sample is considered to be positive for HSV type I if its Ct value is defined in the results grid in JOE/Yellow/HEX channel.
- The sample is considered to be negative if its Ct values are not defined in the results grid in FAM/Green and JOE/Yellow/HEX channels (the fluorescence curves do not cross the threshold line).

Four-channel instrument.

- The sample is considered to be positive for HSV type II if its Ct value is defined in the results grid in FAM/Green channel.
- The sample is considered to be positive for HSV type I if its Ct value is defined in the results grid in JOE/Yellow/HEX channel.
- The sample is considered to be negative if its Ct values are not defined in the results grid in FAM/Green and JOE/Yellow/HEX channels (the fluorescence curves do not cross the threshold line) while Ct value detected in ROX/Orange channel does not exceed Y.
- If Ct value is absent in both FAM/Green and JOE/Yellow/HEX channels as well as in ROX/Orange channel; or Ct value in JOE/Yellow/HEX is higher than X, PCR and detection for that sample should be repeated. If the same result is detected in the second run the analysis should be repeated starting from the extraction stage.

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:

- If Ct values are absent on JOE/Yellow/HEX, FAM/Green and ROX/Orange channels or the Ct value in ROX/Orange channel is higher than Y (for 4-channel instrument), PCR reaction should be repeated. If the same result is achieved, the sample extraction process should be repeated. If the IC signal of this sample was detected normally during any other PCR test, then the PCR test should be repeated.
- If the signal is registered in Negative control of Amplification, it indicates the contamination of 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 also to take

measures to detect and eliminate the source of contamination.

- If no signal is detected for Positive Controls of amplification, it can suggest the incorrect programming of the temperature profile of the applied 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. It is necessary to ensure adequate programming of the Instrument (see 7.2.2.), storage conditions, and check the expiration date of the reagents, and then repeat PCR reaction once again.
- 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 the 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 using iQ5 or iQiCycler Instrument).

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

10. STABILITY AND STORAGE.

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



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

11. SPECIFICATIONS.

11.1. Sensitivity.

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



The claimed analytical features of **AmpliSens® HSV-typing-FRT** PCR kit are guaranteed only when additional reagents kits "DNA-sorb-AM" or "DNA-sorb-B" (manufactured by Federal State Institution of Science Central Research Institute of Epidemiology) are used

11.2. Specificity.

Specificity of **AmpliSens® HSV-typing-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-typing-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 accordance with Federal State Institution of Science "Central Research Institute of Epidemiology" ISO 13485 – certified Quality Management System, each lot of **AmpliSens® HSV-typing-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 complex
	Contains sufficient for <n> tests		Authorized representative in the European Community.
	Consult instructions for use		Caution, consult accompanying documents
	For working with Rotor-Gene™ 3000/6000		For working with iQ5, iQ iCycler
	Positive control		Negative control