AmpliSens® *HSV / CMV*-MULTIPRIME-FRT PCR kit

CE 1023

IVD

For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

REF	Catalogue number	⚠	Caution
LOT	Batch code	Σ	Sufficient for
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	**C+**	Positive control of amplification
FBIS CRIE	Federal Budget Institute of Science "Central Research Institute for Epidemiology"	**IC**	Internal control

1. INTENDED USE

AmpliSens® *HSV / CMV*-MULTIPRIME-FRT PCR kit is an *in vitro* nucleic acid amplification test for simultaneous detection of *herpes simplex virus (HSV)* and *cytomegalovirus* (*CMV*) DNA in clinical material (urogenital, rectal, and oral swabs; urine; saliva; prostate gland secretion; whole blood and cerebrospinal fluid; and exudate of blisters and erosive-ulcerative lesions of skin and mucosa), taken from the persons suspected of herpes virus infection without distinction of form and presence of manifestation, by 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

HSV and *CMV* DNA detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using specific 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 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 / CMV*-MULTIPRIME-FRT PCR kit is a qualitative test that contains the Internal Control (Internal Control-FL (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® *HSV / CMV*-MULTIPRIME-FRT PCR kit uses "hot-start", which greatly reduces the frequency of nonspecifically primed reactions. "Hot-start" is guaranteed by the separation of nucleotides and Taq-polymerase by using chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

The PCR kit contains the system for prevention of contamination by amplicons using the enzyme uracil-DNA-glycosylase (UDG) and deoxyuridine triphosphate. The enzyme UDG recognizes and catalyzes the destruction of the DNA containing deoxyuridine, but has no effect on DNA containing deoxythymidine. Deoxyuridine is absent in the authentic DNA, but is always present in amplicons, because deoxyuridine triphosphate is a part of dNTP mixture in the reagents for the amplification. Due to the deoxyuridine containing contaminating amplicons are sensitive to the destruction by UDG before the DNA-target amplification. So the amplicons cannot be amplified.

The enzyme UDG is thermolabile. It is inactivated by heating at temperature above 50 °C. Therefore, UDG does not destroy the target amplicons which are accumulated during PCR.

The results of amplification are registered in the following fluorescence channels.

Table 1

Channel for fluorophore	FAM	JOE	ROX
DNA-target	*HSV*	*CMV*	Internal Control-FL (IC)
Target gene	*gpB* gene	*Pol* gene	genetically engineered construction

3. CONTENT

AmpliSens® *HSV / CMV*-MULTIPRIME-FRT PCR kit is produced in 1 form:
variant FRT-100 F REF R-V60-F(RG,iQ)-CE.

Variant FRT-100 F includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-1-FL *HSV / CMV*	clear liquid from colorless to light lilac colour	1.2	1 tube
PCR-mix-2-FRT	colorless clear liquid	0.3	2 tubes
Polymerase (TaqF)	colorless clear liquid	0.03	2 tubes
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 (IC)** 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 protocol).

Variant FRT-100 F is intended for 110 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- DNA extraction kit.
- Transport medium.
- Disposable powder-free gloves and a laboratory coat.
- Pipettes (adjustable).
- Disposable tips with aerosol filters (up to 100 µl) in tube racks.
- Tube racks.
- Vortex mixer/desktop centrifuge.
- PCR box.
- Real-time instruments (for example, Rotor-Gene Q (QIAGEN, Germany), CFX96 (Bio-Rad Laboratories, Inc, USA) or equivalent).
- Disposable polypropylene PCR tubes:
  a) thin-walled 0.2-ml PCR tubes with optical transparent domed or flat caps or strips of eight 0.2-ml tubes with optical transparent caps if a plate-type instrument is used;
  b) thin-walled 0.2-ml PCR tubes with flat caps or strips of four 0.1-ml Rotor-Gene PCR tubes if a rotor-type instrument is used.
- Refrigerator for 2–8 °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:

- Temperature in the laboratory room is from 20 to 28 °C, relative humidity is from 15 to 75 %.
- Use sterile pipette tips with aerosol filters and use new tip for every procedure.
- Store all extracted positive material (specimens, controls and amplicons) away from all other 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 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 the PCR kit if the internal packaging was damaged or its appearance was changed.
- Do not use the PCR kit if the transportation and storage conditions according to the Instruction Manual were not observed.
- 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 breathing vapours, 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 if necessary.
- Important note with safety information is available on request.
- The PCR kit is intended for single use for PCR analysis of specified number of samples (see the section "Content").
- The PCR kit is ready for use in accordance with the Instruction Manual. Use the PCR kit strictly for intended purpose.
- 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 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.

Not for use in the Russian Federation

6. SAMPLING AND HANDLING

NOTE: 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 / CMV*-MULTIPRIME-FRT PCR kit is intended for the analysis of DNA extracted with DNA extraction kits from
- urogenital, rectal, and oral swabs;
- urine (a sediment of the first portion of the morning specimen);
- saliva;
- prostate gland secretion;
- whole blood;
- cerebrospinal fluid;
- exudate of blisters and erosive-ulcerative lesions of skin and mucosa.

7. WORKING CONDITIONS

AmpliSens® *HSV / CMV*-MULTIPRIME-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.
- **DNA-sorb-B**, REF K1-2-100-CE (for blood and cerebrospinal fluid samples).
- Other nucleic acid extraction kits recommended by FBIS CRIE (see Guidelines [2]).

The DNA extraction of each test sample is carried out in the presence of **Internal Control-FL (IC).**

NOTE: Extract DNA according to the manufacturer's protocol.

8.2. Preparing PCR

8.2.1 Preparing tubes for PCR

The total reaction volume is **25 µl**, the volume of DNA sample is **10 µl**.
1. Thaw the tube with **PCR-mix-2-FRT.** Vortex the tubes with **PCR-mix-1-FL *HSV / CMV,* PCR-mix-2-FRT, polymerase (TaqF)** then centrifuge briefly (1–2 s). Make sure there are no drops on the walls of the tubes.
   Take the required number of the tubes for amplification of DNA from test and control samples.
2. For N reactions (including 2 controls of amplification), add to a new tube:
   10*(N+1) µl of PCR-mix-1-FL *HSV / CMV*;
   5.0*(N+1) µl of PCR-mix-2-FRT;
   0.5*(N+1) µl of polymerase (TaqF).
   Mix the prepared mixture and sediment the drops by short centrifugation (1-2 s). Transfer **15 µl** of the prepared mix to each tube.
3. Using tips with aerosol filter, add **10 µl** of **DNA samples** obtained from test or control samples at the DNA extraction stage.
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 **the sample extracted from the Negative Control reagent** to the tube labeled C– (Negative Control of Extraction).

8.2.2. Amplification

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

Table 2

«AmpliSens-1M» program

	Rotor-type instruments[1]			Plate-type instruments[2]		
Step	**Temperature, °C**	**Time**	**Cycles**	**Temperature, °C**	**Time**	**Cycles**
1	**95**	**15 min**	**1**	**95**	**15 min**	**1**
2	**95**	**20 s**	**5**	**95**	**20 s**	**5**
	60	**20 s**		**60**	**20 s**	
	72	**15 s**		**72**	**15 s**	
3	**95**	**20 s**	**40**	**95**	**20 s**	**40**
	60	**20 s Fluorescence detection**		**60**	**30 s Fluorescence detection**	
	72	**15 s**		**72**	**15 s**	

Fluorescent signal is detected in the channels for the FAM, JOE and ROX 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 *Important Product Information Bulletin* and Guidelines [2].
3. Insert the tubes into the reaction module of the instrument.
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 three channels:
- The signal of the *HSV* DNA amplification product is detected in the channel for the FAM fluorophore;
- The signal of the *CMV* DNA amplification product is detected in the channel for the JOE fluorophore;
- The signal of the IC amplification product is detected in the channel for the ROX 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.
Principle of interpretation is the following:
- ***HSV*** DNA is **detected** if the *Ct* value is determined in the results grid in the channel for the FAM fluorophore. Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.
- ***CMV*** DNA is **detected** if the *Ct* value is determined in the results grid in the channel for the JOE fluorophore. Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.
- ***HSV*** and ***CMV*** DNA is **not detected** in a sample if *Ct* value is not determined in the results grid (the fluorescence curve does not cross the threshold line) in the channels for the FAM and JOE fluorophores and if the *Ct* value determined in the results grid in the channel for the ROX fluorophore does not exceed the specified boundary *Ct* value.
- The analysis result is considered to be **invalid** if the *Ct* value is not determined (absent) or greater than the specified boundary *Ct* value in the channel for the ROX fluorophore and in the channels for the FAM and JOE fluorophores. In such cases the PCR analysis should be repeated.

NOTE: Boundary *Ct* values are specified in the *Important Product Information Bulletin* enclosed to the PCR kit. See also Guidelines [2]

The result of the analysis is considered reliable only if the results obtained for both Positive and Negative Controls of amplification as well as for the Negative Control of extraction are correct (see Table 3).

Table 3

Results for controls

Control	Stage for control	*Ct* value in the channel for fluorophore	
		FAM, JOE	ROX
C–	DNA extraction	Absent	<boundary value
NCA	PCR	Absent	Absent
C+	PCR	<boundary value	<boundary value

10. TROUBLESHOOTING

Results of analysis are not taken into account in the following cases:
1. If the *Ct* value determined for the Positive Control of Amplification (C+) in the channels for the FAM and/or JOE fluorophores is greater than the boundary *Ct* value or absent, the amplification should be repeated for all samples in which Ct value is absent in the channels for the FAM and/or JOE fluorophores respectively.
2. If the *Ct* value is determined for the Negative Control of Amplification (NCA) and/or Negative Control of Extraction (C–) in the channels for the FAM or JOE fluorophores the PCR analysis should be repeated for all samples in which *Ct* value was determined in the channels for the FAM and/or JOE fluorophores respectively.

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

11. TRANSPORTATION

AmpliSens® *HSV / CMV*-MULTIPRIME-FRT PCR kit should be transported at 2–8 ºC for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens® *HSV / CMV*-MULTIPRIME-FRT** PCR kit are to be stored at 2–8 °C when not in use (except for polymerase (TaqF) and PCR-mix-2-FRT). All components of the **AmpliSens® *HSV / CMV*-MULTIPRIME-FRT** PCR kit are stable until the labeled expiration date. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

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

NOTE: PCR-mix-1-FL *HSV / CMV* is to be stored away from light.

13. SPECIFICATIONS

13.1. Sensitivity

Clinical material	Nucleic acid extraction kit	Pathogen	Sensitivity, GE/ml[3]
Urogenital swabs[4]	**DNA-sorb-AM**	*HSV*	$1x10^3$
		CMV	$1x10^3$
Urine [5]	**DNA-sorb-AM**	*HSV*	N/A
		CMV	$2x10^3$

The analytical sensitivity for each microorganism is preserved in the presence of high DNA concentrations of other analyte microorganism (up to10^9 GE/ml).

[1] For example, Rotor-Gene Q or equivalent.
[2] For example, CFX 96 or equivalent.
[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, REF 987-CE) or **Transport Medium with Mucolytic Agent** (REF 953-CE).
[5] Pretreatment is required.

13.2. Specificity

The analytical specificity of **AmpliSens® *HSV / CMV*-MULTIPRIME-FRT** PCR kit is ensured by the selection of specific primers and probes as well as stringent reaction conditions. The primers and probes were checked for possible homologies to all sequences published in gene banks by sequence comparison analysis.

The specificity was proved on the panel of DNA samples of the following microorganisms: *CMV; EBV; HHV* types 6 and 7*; HPV; Gardnerella vaginalis; Lactobacillus* spp.*; Escherichia coli; Staphylococcus aureus; Streptococcus pyogenes; Streptococcus agalactiae; Candida albicans; Mycoplasma hominis; Ureaplasma urealyticum; Ureaplasma parvum; Mycoplasma genitalium; Neisseria flava; Neisseria subflava; Neisseria sicca; Neisseria mucosa; Neisseria gonorrhoeae; Chlamydia trachomatis; Treponema pallidum; Trichomonas vaginalis; Toxoplasma gondii.* Nonspecific responses were absent while testing this panel as well as human DNA samples.

The clinical specificity of **AmpliSens® *HSV / CMV*-MULTIPRIME-FRT** PCR kit was confirmed in laboratory clinical trials.

13.3. Diagnostic sensitivity

The diagnostic sensitivity of the **AmpliSens® *HSV / CMV*-MULTIPRIME-FRT** PCR kit is 99 %.

13.4. Diagnostic specificity

The diagnostic specificity of the **AmpliSens® *HSV / CMV*-MULTIPRIME-FRT** PCR kit is 99.3 %.

14. REFERENCES

1. Handbook “Sampling, Transportation, and Storage of Clinical Material for PCR Diagnostics”, developed by Federal Budget Institution of Science “Central Research Institute for Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being.
2. Guidelines “Real-Time PCR Detection of STIs and Other Reproductive Tract Infections”, developed by Federal Budget Institution 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 the **AmpliSens® *HSV / CMV*-MULTIPRIME-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
23.06.11 LA	Cover page, text	The name of Institution was changed to Federal Budget Institution of Science “Central Research Institute for Epidemiology”
24.09.15 PM	1. Intended use	It is specified that clinical material is taken from the persons suspected of herpes virus infection without distinction of form and presence of manifestation
	3. Content, Footer	REF R-V60(iQ)-CE was deleted
	9. Data analysis 10. Troubleshooting	The section was rewritten
	13. Specifications	The list of microorganisms, on which the specificity was proved, was increased. Diagnostic sensitivity and specificity was added
	Through the text	Corrections in accordance with the template
16.12.15 ME	3. Content	Number of forms was corrected from 3 to 2
23.10.17 ME	Text	Corrections according to the template
	8.1. DNA extraction	Information about controls of extraction was added
	8.2.2. Amplification	The amplification program was changed from AmpliSens-1 to AmpliSens-1M
29.12.17 PM	3. Content	The color of the reagent was specified
05.12.18 PM	2. Principle of PCR detection	The table with targets and the information about the enzyme UDG were added
	Through the text	The text formatting was changed
27.02.20 PM	Footer	The phrase “Not for use in the Russian Federation” was added
26.10.20 MM	Footer, 3. Content	The information about variant FRT REF R-V60(RG)-CE was deleted
01.03.21 MM	—	The name, address and contact information for Authorized representative in the European Community was changed

AmpliSens®

EC REP

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