CE 1023

IVD

For Professional Use Only

AmpliSens® *HSV / CMV*-MULTIPRIME-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

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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 materials (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) 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

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 (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 separation of nucleotides and Taq-polymerase by using a wax layer or a chemically modified polymerase (TaqF). Wax melts and reaction components mix only at 95ºC. Chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

3. CONTENT

AmpliSens® *HSV / CMV*-MULTIPRIME-FRT PCR kit is produced in 3 forms:

AmpliSens® *HSV / CMV*-MULTIPRIME-FRT PCR kit variant FRT (for use with RG)

REF R-V60(RG)-CE.

AmpliSens® *HSV / CMV*-MULTIPRIME-FRT PCR kit variant FRT (for use with iQ)

REF R-V60(iQ)-CE

AmpliSens® *HSV / CMV*-MULTIPRIME-FRT PCR kit variant FRT-100 F (for use with RG or iQ) **REF** R-V60-F(RG,iQ)-CE.

AmpliSens® *HSV / CMV*-MULTIPRIME-FRT PCR kit variant FRT includes:

Reagent	*Description*	*Volume, ml*	*Quantity*
PCR-mix-1-FL *HSV / CMV* ready-to-use single-dose test tubes (*under wax)*	colorless clear liquid	0.01	110 tubes of 0.2 ml volume
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 or DNA-sorb-B, **REF** K1-2-100-CE protocols).

AmpliSens® *HSV / CMV*-MULTIPRIME-FRT PCR kit variant FRT is intended for 110 reactions (including controls).

AmpliSens® *HSV / CMV*-MULTIPRIME-FRT PCR kit variant FRT-100 F includes:

Reagent	*Description*	*Volume, ml*	*Quantity*
PCR-mix-1-FL *HSV / CMV*	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 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 during the DNA extraction procedure directly to the sample/lysis mixture (see DNA-sorb-AM **REF** K1-12-100-CE or DNA-sorb-B, **REF** K1-2-100-CE protocols).

AmpliSens® *HSV / CMV*-MULTIPRIME-FRT PCR kit variant FRT-100 F is intended for 110 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- DNA extraction kit.
- Transport medium.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Disposable tips with aerosol barriers (up to 100 µl) in tube racks.
- Tube racks.
- Vortex mixer/desktop centrifuge.
- PCR box.
- Personal thermocyclers (for example, Rotor-Gene 3000 or Rotor-Gene 6000 (Corbett Research, Australia); Rotor-Gene Q (Qiagen, Germany), iCycler 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 mucous membranes. If skin, eyes, or mucous

membranes contact, immediately flush with water and 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 Areas. 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 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 that the following nucleic acid extraction kits are used:

- 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])

Extract DNA according to the instructions provided by the manufacturer.

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 the tubes with **PCR-mix-1-FL *HSV / CMV*** and wax for amplification of DNA from test 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 *HSV / CMV.*

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.** 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.
2. Prepare the required number of the tubes for amplification of DNA from test and control samples.
3. For N reactions (including 2 controls of amplification), mix in 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).

Stir the prepared mixture and then centrifuge briefly (1–2 s). Make sure there are no drops on the walls of the tubes. Transfer **15 µl** of the prepared mix to each tube.

Steps 3 and 4 are carried out in both variants.

4. Using tips with aerosol barrier add **10 µl** of **DNA** obtained from test or control samples at the DNA extraction stage into prepared tubes.
5. 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, extracted from Negative Control** to the tube labeled C– (Negative Control of Extraction).

8.2.2. Amplification

1. Program the thermocycler according to **Manufacturer's manual**, **Guidelines** and Table 1.

Table 1

AmpliSens-1 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**	5 s	5	**95**	5 s	5
	60	20 s		**60**	20 s	
	72	15 s		**72**	15 s	
3	**95**	5 s	40	**95**	5 s	40
	60	20 s *Fluorescence detection*		**60**	30 s *Fluorescence detection*	
	72	15 s		**72**	15 s	

Fluorescent signal is detected in FAM, JOE and ROX channels (other channels can be used during simultaneous analysis with other PCR kits)

2. Insert the tubes into the device reaction module cells.
3. Set the amplification program with fluorescence detection.
4. After the completion of the amplification program, analyze, and record the results.

9. DATA ANALYSIS

The fluorescent signal intensity is detected in three channels:

HSV DNA is detected in the FAM channel; *CMV* DNA is detected in the JOE channel; Internal Control DNA is detected in the ROX fluorescence channel.

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

Principle of interpretation:

1. The sample is considered to be positive for the *HSV* DNA if its Ct value is defined in the results grid in the FAM channel (the fluorescence curve should cross the threshold line in the region of significant fluorescence increase).
2. The sample is considered to be positive for the *CMV* DNA if its Ct value is defined in the results grid in the JOE channel (the fluorescence curve should cross the threshold line in the region of significant fluorescence increase).
3. The sample is considered to be negative for *HSV* and *CMV* DNA if its Ct value is not defined in the results grid (the fluorescence curve does not cross the threshold line) in FAM and JOE channels and the Ct value does not exceed the boundary value in the results grid in the ROX channel.
4. The analysis result is considered to be invalid if the Ct value is not defined in the results

[1] For example, Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q, or equivalent.
[2] For example, iCycler, iQ5, Mx3000P, Mx3000, DT-96, or equivalent.

grid (the fluorescence curve does not cross the threshold line) in the ROX channel and the Ct value in the results grid in the FAM and JOE channels exceeds the threshold value. In such cases PCR should be repeated.

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 2).

Table 2

Results for controls

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

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

10. TROUBLESHOOTING

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

- If the Ct value for the positive control of PCR (C+) in FAM and/or JOE channel is absent or exceeds the boundary Ct value, it is necessary to repeat PCR of all samples in which the boundary Ct value in the FAM and/or JOE channel was not detected.
- If the Ct value for the negative control of extraction (C–) or/and for the negative control of amplification (NCA) in the FAM or/and JOE channel is detected, it is necessary to repeat analysis of all samples in which a Ct value in the FAM and/or JOE channel was detected.

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 °C for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens® *HSV / CMV*-MULTIPRIME-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® *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.

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 *HSV* / *CMV* is to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

Analytical Sensitivity of **AmpliSens® *HSV* / *CMV*-MULTIPRIME-FRT** PCR kit is specified in the table below.

Clinical material	Nucleic acid extraction kit	Pathogen	Sensitivity, GE/ml[3]
Urogenital swabs[4]	DNA-sorb-AM	*HSV*	10^3
		CMV	10^3
Urine [5]	DNA-sorb-AM	*HSV*	N/A
		CMV	$2x10^3$

13.2. Specificity

The analytical specificity of **AmpliSens® *HSV* / *CMV*-MULTIPRIME-FRT** PCR kit is ensured by selection of specific primers and probes as well as by 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. There were not nonspecific test responses during examination of a human DNA as well as a DNA panel of the following microorganisms: *Gardnerella vaginalis, Lactobacillus* spp., *Escherichia coli, Staphylococcus* spp., *Streptococcus* spp., *Candida albicans, Mycoplasma hominis, Ureaplasma urealyticum, Ureaplasma parvum, Mycoplasma genitalium, Chlamydia trachomatis, Neisseria* spp., *Neisseria gonorrhoeae, Trichomonas vaginalis, Treponema pallidum, Toxoplasma gondii, HSV* 1 and 2, *CMV, HPV.*

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

[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 Agent (**REF** 953-CE).

[5] Pretreatment is required.

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, Moscow, 2008.
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 Institution of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Quality Management System, each lot of **AmpliSens®** ***HSV / CMV*-MULTIPRIME-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code		Sufficient for
IVD	*In vitro* diagnostic medical device		Expiration Date
VER	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
EC REP	Authorised representative in the European Community	**C+**	Positive control of amplification
RG	For working with Rotor-Gene 3000/6000 (Corbett Research)	**IC**	Internal control
FBIS CRIE	Federal Budget Institution of Science "Central Research Institute for Epidemiology"	**iQ**	For working with iQ5, iCycler iQ (Bio-Rad)

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”