CE 1023

IVD

For Professional Use Only

AmpliSens® *CMV*-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

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1. INTENDED USE

AmpliSens® ***CMV*****-FRT** PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of human cytomegalovirus (*CMV*) DNA in the clinical materials (urogenital swabs, urine samples, saliva, whole human blood) 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

CMV DNA detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using special 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 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®** ***CMV*****-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®** ***CMV*****-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 95 ºC. Chemically modified polymerase (TaqF) is activated by heating at 95 ºC for 15 min

3. CONTENT

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

AmpliSens® *CMV*-FRT PCR kit variant FRT (for use with RG) **REF** R-V7(RG)-CE.

AmpliSens® *CMV*-FRT PCR kit variant FRT (for use with iQ) **REF** R-V7(iQ)-CE.

AmpliSens® *CMV*-FRT PCR kit variant FRT-100 F (for use with RG or iQ) **REF** R-V7-F(RG,iQ)-CE.

AmpliSens® *CMV*-FRT PCR kit variant FRT includes:

Reagent	*Description*	*Volume (ml)*	*Quantity*
PCR-mix-1-FL *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 protocol).

AmpliSens® *CMV*-FRT PCR kit is intended for 110 reactions, including controls.

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

Reagent	*Description*	*Volume (ml)*	*Quantity*
PCR-mix-1-FL *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 protocol).

AmpliSens® *CMV*-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.
- Adjustable automatic pipettes (from 5 to 20 µl, when using of PCR kit variant FRT-100

F - from 5 to 20 µl and from 20 to 200 µl).

- 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-ml for variant FRT and 0.2- or 0.1-ml for variant FRT-100 F; 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 other suitable disinfectant.
- Avoid contact with the skin, eyes, and mucous membranes. If skin, eyes, and 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 is described in manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

AmpliSens® *CMV*-FRT PCR kit is intended for the analysis of DNA extracted by DNA extraction kits from scrapes from mucous membranes of urogenital tract, urine samples, saliva and whole human blood.

7. WORKING CONDITIONS

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

8. PROTOCOL

8.1. DNA Extraction

It's recommended to use the following nucleic acid extraction kits:

- DNA-sorb-AM, **REF** K1-12-100-CE.
- Other nucleic acid extraction kits, recommended by Federal Budget Institution of Science "Central Research Institute for Epidemiology" of Federal Service for Surveillance on Consumers' Rights Protection and Human Well-Being (see **Guidelines**).

Extract DNA according to the manufacturer's instructions.

8.2. PCR with real-time hybridization-fluorescence detection

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 *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 *CMV.***

Variant FRT-100 F

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

1. Prepare the required number of the tubes for amplification of DNA from test and

control samples.

2. For carrying out N reactions (including 2 controls), mix in a new tube: **10*(N+1) µl of PCR-mix-1-FL *CMV*, 5.0*(N+1) µl of PCR-mix-2-FRT** and **0.5*(N+1) µl of polymerase (TaqF).** Mix the content of the tube by vortexing and then centrifuge shortly. Transfer **15 µl** of the 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** obtained from test or control samples at the DNA extraction stage into the prepared tubes.
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).

8.2.2. Amplification

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

Table 1

«AmpliSens-1» program

	Rotor-type devices (for example, Rotor-Gene 3000/6000, Rotor-Gene Q, or equivalent)			**Plate-type devices** (for example, iCycler iQ or iQ5, Mx3000P, Mx3000, or equivalent)		
Cycle	Temperature, °C	Time	Repeats	Temperature, °C	Time	Repeats
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	

2. Insert the tubes into the reaction module cells of the instrument.
3. Adjust the fluorescence channel sensitivity according to Important Product Information Bulletin.
4. Run the amplification program with fluorescence detection.
5. Analyze results after the amplification program is completed.

9. DATA ANALYSIS

The fluorescent signal intensity is detected in two channels:

– The signal from the *CMV* DNA amplification product is detected in the FAM channel;

The signal from the Internal Control amplification product is detected in the JOE/Yellow/HEX channel.

Interpretation of results

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

Principle of interpretation:

- *CMV* DNA is **detected** in a sample if its Ct value is present in the FAM channel. The fluorescence curve should cross the threshold line in the area of exponential fluorescence growth.
- *CMV* DNA is **not detected** in a sample if its Ct value is absent in the FAM channel (fluorescence curve does not cross the threshold line) and the Ct value in the JOE channel is less than the specified boundary Ct value.
- The result is **invalid** if the Ct value of a sample in the FAM channel is absent while the Ct value in the JOE channel is either absent or greater than the boundary Ct value specified. It is necessary to repeat the PCR analysis of such samples (see Table 2).

Table 2

Results for controls

Control	Stage for control	Ct value on channel		Interpretation
		FAM	JOE	
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 of the positive control of amplification (C+) in the FAM channel is absent or greater than the boundary Ct value, repeat PCR analysis of all samples in which *CMV* DNA was not found.
- If a Ct value is detected for the Negative Control of extraction (C–) and/or Negative Control of amplification (NCA) in the FAM channel, repeat PCR analysis for all samples in which *CMV* DNA was found starting from the DNA extraction stage.

If you have any further questions or if encounter problems, please contact our Authorized

representative in the European Community.

11. TRANSPORTATION

AmpliSens® *CMV*-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® *CMV*-FRT** PCR kit (except for Polymerase (TaqF) and PCR-mix-2-FRT) are to be stored at 2–8 ºC when not in use. All components of the **AmpliSens® *CMV*-FRT** PCR kit are to be stable until 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 ºC when not in use.

PCR-mix-1-FL *CMV* is to be stored away from light.

13. SPECIFICATIONS

13.1. Sensitivity

Analytical Sensitivity of **AmpliSens® *CMV*-FRT** PCR kit is the following:

Clinical material	Transport medium	Nucleic acid extraction kit	Sensitivity, GE/ml*
Urogenital swabs	Transport Medium for Swabs or Transport Medium with Mucolytic	DNA-sorb-AM	10^3
Urine (pretreatment is required)	–	DNA-sorb-AM	$2x10^3$

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

13.2. Specificity

The analytical specificity of **AmpliSens® *CMV*-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 published in gene banks by sequence comparison analysis. The clinical specificity of **AmpliSens® *CMV*-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 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® *CMV*-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

REF	Catalogue number		Sufficient for
LOT	Batch code		Expiration Date
IVD	*In vitro* diagnostic medical device		Consult instructions for use
VER	Version		Keep away from sunlight
	Temperature limitation	**NCA**	Negative control of amplification
	Manufacturer	**C–**	Negative control of extraction
	Date of manufacture	**C+**	Positive control of amplification
EC REP	Authorised representative in the European Community	**IC**	Internal control
	Caution		

List of Changes Made in the Instruction Manual

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