



For *in Vitro* Diagnostic Use

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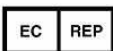
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AmpliSens[®] CMV-FRT

PCR kit

Instruction Manual

AmpliSens[®]



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1. INTENDED USE.

AmpliSens® CMV-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection and quantification 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 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® CMV-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® CMV-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® CMV-FRT PCR kit is produced in 3 forms:

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

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

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

AmpliSens® CMV-FRT PCR kit variant FRT includes:

Reagent	Description	Volume (ml)	Quantity
PCR-mix-1-FL CMV	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**	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® 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**	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® CMV-FRT PCR kit is intended for 110 reactions, including controls.

4. ADDITIONAL REQUIREMENTS.

- DNA isolation kit.
- Transport medium.
- Disposable powder-free gloves and laboratory coat.

- Automated pipettors (dosers) of variable volumes (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) iQ5 (BioRad, USA), Mx3000P (Stratagene, USA) or equivalent).
- Disposable polypropylene microtubes for PCR of 0.2 or 0.1 ml volume - when using of PCR kit variant FRT-100 F (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® CMV-FRT PCR kit is intended for the analysis of DNA extracted by DNA isolation kits from scrapes from mucous membranes of urogenital tract, urine samples, saliva and whole human blood.

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.
- Other nucleic acid extraction kits, recommended by Federal State Institution of Science "Central Research Institute of Epidemiology" of Federal Service for Surveillance on Consumers' Rights Protection and Human Well-Being (see **Guidelines**).



Carry the DNA isolation according to the manufacturer's instructions.

7.2. PCR with real-time hybridization-fluorescence detection.

7.2.1 Preparing tubes for PCR.

Variant FRT

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**.

Variants FRT-100 F and FRT-1000 F

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 of 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 vortex mixer, 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** obtained from test or control samples at the DNA extraction stage into prepared tubes.
4. Carry 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).

7.2.2. Amplification

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

Table 1

«AmpliSens-1» program

Cycle	Rotor type devices, for example, «Rotor-Gene» 3000/6000, «Rotor-Gene Q» or equivalent			Flatbed devices, for example, «iQ», «iCycler», «iQ5», «Mx3000P», «Mx3000» or equivalent		
	Temperature, °C	Time	Cycle index	Temperature, °C	Time	Cycle index
1	95	15 min	1	95	15 min	1
2	95	5 sec	5	95	5 sec	5
	60	20 sec		60	20 sec	
	72	15 sec		72	15 sec	
3	95	5 sec	40	95	5 sec	40
	60	20 sec <i>Fluorescence detection</i>		60	30 sec <i>Fluorescence detection</i>	
	72	15 sec		72	15 sec	

2. Set the tubes into the device reaction module cells.
3. Set the amplification program with fluorescence detection.
4. After amplification program run finishing make analysis and record of results.

8. DATA ANALYSIS.

The results - fluorescence curve on two channels - are interpreted by used device software for PCR with real-time hybridization-fluorescence detection. Internal Control is detected in the JOE

fluorescence channel, *CMV* DNA is detected in the FAM fluorescence channel.

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

1. The sample is considered to be positive for *CMV* DNA if its Ct value is defined in the results grid in FAM channel.
2. The sample is considered to be negative for *CMV* DNA if its Ct value is not defined in the results grid (the fluorescence curve does not cross the threshold line) in FAM channel and in the results grid in the JOE channel the Ct value doesn't exceed threshold value.
3. The analysis result is considered to be invalid if the Ct value is not defined in the results grid (the fluorescence curve does not cross the threshold line) in FAM channel and in the results grid in the JOE channel the Ct value exceeds threshold value. In such cases PCR should be repeated.

Result of the analysis is considered to be reliable only if both Positive and Negative Controls of amplification as well as Negative Control of extraction are passed (see table 2).

Table 2

Results for controls

Control	Stage for control	Ct value on channel		Interpretation
		FAM	JOE	
C-	DNA isolation	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**.

9. TROUBLESHOOTING.

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

- If for positive control of PCR (C+) Ct value in FAM channel doesn't exceed threshold value of positive result it's necessary to repeat the amplification and detection for all samples where *CMV* DNA was not determined.
- If for negative control of extraction (C-) or/and for negative control of amplification (NCA) Ct value in FAM channel exceeds threshold value of positive result it's necessary to repeat PCR analysis for all samples where *CMV* DNA was determined beginning from DNA extraction stage.

10. 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 the temperature between 2 °C and 8 °C, when not in use. All components of the **AmpliSens® CMV-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



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

11. SPECIFICATIONS.

11.1. Sensitivity.

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

Clinical material	Nucleic acid extraction kit	Sensitivity, GE/ml
Urogenital swabs	DNA-sorb-AM	10 ³ GE/ml ¹
Urine ²	DNA-sorb-AM	2x10 ³ GE/ml

11.2. Specificity.

Specificity of **AmpliSens® CMV-FRT** PCR kit is assured by selection of specific primers and probes, as well as the selection of strict 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® CMV-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.
- Guidelines "Real-time PCR detection of STIs and other reproductive tract infections",

¹ Cervical, urethral scrapes (swabs) are to be placed into the Transport medium for swabs (**REF** 956, 987) or Transport medium with mucolytic (**REF** 952, 953).

² Treatment is required.

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.

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® CMV-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 *in Vitro* Diagnostic Use



Version



Catalogue number



Caution, consult accompanying documents



Contains sufficient for <n> tests



Negative Control of Amplification



Consult instructions for use



Negative control of Extraction



Positive Control of Amplification