



For *in Vitro* Diagnostic Use

AmpliSens® HHV6-screen-titre-FRT

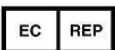
PCR kit

Instruction Manual



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1. INTENDED USE.

AmpliSens® HHV6-screen-titre-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection and quantitation of *Human Herpes Virus* type 6 DNA in the clinical materials (whole blood, umbilical cord blood, white blood cells, biopsy material, saliva, throat washes and swabs) by means of real-time hybridization-fluorescence detection.

2. PRINCIPLE OF PCR DETECTION.

Human Herpes Virus type 6 detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using special *Human Herpes Virus* 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. The real-time PCR monitoring of the fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening of the reaction tubes after the PCR run. AmpliSens® HHV6-screen-titre-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 chemically modified polymerase (TaqF) that activates by heating at 95°C for 15 min. AmpliSens® HHV6-screen-titre-FRT PCR kit is based on:

- extraction of total DNA from cell suspension (whole blood, white blood cells, biopsy material, saliva, throat washes and swabs);
- simultaneous real time amplification (multiplex-PCR) of HHV6 pol-gene fragment and β-globin gene fragment, that is used as endogenous internal control. Result of amplification of HHV6 DNA is registered in JOE/Yellow fluorescence channel; Internal Control is registered in FAM/Green fluorescence channel.

DNA-target selected as endogenous internal control is the fragment of human genome and must be present in a blood DNA sample in sufficient quantity no less than 20,000 genomes per sample (DNA from 10,000 cells). In case of inadequate storage, cells damage and DNA degradation will take place. Failure in DNA extraction can lead to significant losses of DNA and presence of inhibitors in purified DNA sample. Therefore, not only does endogenous internal control allow monitoring of the test stages (DNA extraction and PCR conducting) but also to assess the adequacy of clinical material collection and storage.

For estimation of HHV6 DNA copies per standard amount of human cells (genomes), quantitative calibrators are used. They make it possible to estimate the number of HHV6 DNA copies as well as β-globin gene DNA (Glob) in a reaction tube.

HHV6 DNA quantity calculation is performed as follows:

$$C_{HHV6 \text{ DNA per } 10^5 \text{ cells}} = C_{HHV6 \text{ DNA}} / C_{glob} \times 2 \cdot 10^5$$

$C_{HHV6 \text{ DNA}}$ – quantity of HHV6 DNA copies in the PCR-sample;

C_{glob} - quantity of human DNA copies in the PCR-sample.

To represent the relative concentration of HHV6 DNA in copies per standard blood cell quantity (for example, per 10⁵) the following coefficient is used:

$$10^5 \text{ cells} = 2 \cdot 10^5 \text{ human genomes}$$

3. CONTENT.

AmpliSens® HHV6-screen-titre-FRT PCR kit is produced in 1 form:

AmpliSens® HHV6-screen-titre-FRT variant screen-titre-FRT (for use with RG, iQ, Mx) **REF** R-V10-T(RG,iQ,Mx)-CE

AmpliSens® HHV6-screen-titre-FRT PCR kit includes:

Reagent		Description	Volume (ml)	Amount
PCR-mix-1-FRTHHV-6/Glob		colorless, clear liquid	0.6	2 tubes
PCR-mix-2-FRT		colorless, clear liquid	0.3	2 tubes
Polymerase (TaqF)		colorless, clear liquid	0.03	2 tubes
Positive Control DNA HHV-6 and human DNA		colorless, clear liquid	0.2	1 tube
DNA-buffer		colorless, clear liquid	0.5	1 tube
DNA-calibrators HHV6+Glob	HG1	colorless, clear liquid	0.06	1 tube
	HG2	colorless, clear liquid	0.06	1 tube
	HG3	colorless, clear liquid	0.06	1 tube
Negative Control (C-)*		clear liquid of stramineous color	1.6	3 tubes

* must be used in the isolation procedure as Negative Control of Extraction.

AmpliSens® HHV6-screen-titre-FRT PCR kit is intended for 120 reactions, including controls.

4. ADDITIONAL REQUIREMENTS.

- DNA isolation kit.
- Disposable powder-free gloves.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol filters 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 iQ iCycler (BioRad, USA) Instrument.
- Disposable polypropylene microtubes for PCR with 0.5 (0.2) ml capacity.
- Refrigerator for 2–8 °C.
- Deep-freezer with temperature below minus 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 filters 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 an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats and eye protection when handling specimens and reagents. 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 specimens and unused reagents in accordance with local regulations.
- Specimens 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 sample or reagent contact with the skin, eyes and mucose membranes. If any of these solutions come into contact, rinse immediately with water and seek medical advice immediately.
- 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.
- Workflow in the laboratory must proceed in a unidirectional manner, beginning in the Extraction Area and moving 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 of biological material samples for PCR-analysis, transportation and storage are described in manufacturer's handbook [1]. It is recommended that this handbook is read before starting of the work.

AmpliSens® HHV6-screen-titre-FRT PCR kit is intended for the analysis of DNA extracted with DNA isolation kits from:

- whole peripheral and umbilical cord blood
- white cells of peripheral or/and umbilical cord blood
- biopsy material of the internal organs
- saliva
- throat washes and swabs

7. PROTOCOL.

7.1. DNA Isolation

It's recommended using of the following nucleic acid extraction kits:

Extraction kit	REF	Clinical material for DNA extraction
DNA-sorb-AM RIBO-prep DNA-sorb-B	K1-2-50-CE K1-12-100-CE K2-9-Et-100-CE	Saliva, throat washes and swabs
DNA-sorb-B DNA-sorb-C	K1-2-50-CE K1-6-50-CE	Biopsy material of the internal organs
DNA-sorb-B RIBO-prep	K1-2-50-CE K2-9-Et-100-CE	White cells of peripheral or/and umbilical cord blood
DNA-sorb-B	K1-2-50-CE	Whole peripheral and umbilical cord blood



Carry out the DNA isolation according to the manufacturer instruction.



White blood cells should be treated with "Hemolytic" REF 137 before adding the lysis solution (both for RIBO-prep and for DNA-sorb-B protocols). To do this, add 1.0 ml of "Hemolytic" and 0.25 ml of whole blood in 1.5 ml tube. Vortex carefully. Centrifuge (2 min, 8,000 rpm). Remove the supernatant, leaving 100 µl of the liquid above the sediment. Cells sediment should be white after washing. Presence of little pink deposits is acceptable.

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.

1. Prepare the reaction mix, calculating per one reaction:

- 10 µl PCR-mix-1-FRT HHV-6 /Glob

- 5.0 µl PCR-mix-2-FRT

- 0.5 µl polymerase (TaqF)

Refer to Appendix 1 for calculation of reaction volumes. Take into account that analysis of even a single DNA sample should include five amplification stage controls: three DNA calibrators; negative and positive controls of PCR.

It is recommended to prepare reaction mix for even numbers of reactions to ensure precise reagents dispensing.

2. Prepare required number of tubes or stripes for amplification of DNA of clinical and control samples, including controls.
3. Add 15 µl of prepared reaction mix into each tube.
4. Using tips with aerosol filter add 10 µl of DNA samples, obtained from clinical or control samples at the stage of DNA extraction.
5. Carry out the control amplification reactions:

For qualitative test:

NCA Add 10 µl of DNA-buffer to the tube for Negative Control of Amplification (NCA).

C+ Add 10 µl of Positive Control DNA HHV-6 and human DNA in the tube for Positive Control of Amplification.

For quantitative test:

NCA Add 10 µl of DNA-buffer to the tube for Negative Control of Amplification (NCA).

C+ Add 10 µl of Positive Control DNA HHV-6 and human DNA in the tube for Positive Control of Amplification.

HG1 Add 10 µl of corresponding DNA-calibrator HHV6+Glob (Yellow/JOE/HEX) to the tube.

HG2 Add 10 µl of corresponding DNA-calibrator HHV6+Glob (Yellow/JOE/HEX) to the tube.

HG3 Add 10 µl of corresponding DNA-calibrator HHV6+Glob (Yellow/JOE/HEX) to the tube.

7.2.2. Amplification

Program the Real-time instrument according to manufacturer's manual.

7.2.2.1. For Rotor-Gene™ run the AmpliSens-1 RG amplification program (see table 2).

Table 2

AmpliSens-1 RG amplification program

Step	Temperature, °C	Time	Fluorescence detection	Repeats
Hold	95	15 min	–	1
	95	5 sec	–	
Cycling	60	20 sec	–	5
	72	15 sec	–	
	95	5 sec	–	
Cycling2	95	5 sec	–	40
	60	20 sec	FAM/Green, JOE/Yellow	
	72	15 sec	–	

See Appendix 2 for settings.

7.2.2.2. For iQ iCycler™, iQ™ run the AmpliSens-1 iQ amplification program (see table 3).

Table 3

AmpliSens-1 iQ amplification program

Step	Temperature, °C	Time	Fluorescence detection	Repeats
1	95 °C	15 min	–	1
2	95 °C	5 sec	–	5
	60 °C	20 sec	–	
	72 °C	15 sec	–	
3	95 °C	5 sec	–	40
	60 °C	30 sec	FAM, HEX	
	72 °C	15 sec	–	

See Appendix 3 for settings.

7.2.2.3. For Mx3000P™ and Mx3005P™ run the AmpliSens-1 Mx amplification program (see table 4).

Table 4

AmpliSens-1 Mx amplification program

Step	Temperature, °C	Time	Fluorescence detection	Repeats
1	95 °C	15 min	–	1
2	95 °C	5 sec	–	5
	60 °C	20 sec	–	
	72 °C	15 sec	–	
3	95 °C	5 sec	–	40
	60 °C	30 sec	FAM, JOE/HEX	
	72 °C	15 sec	–	

See Appendix 4 for settings.



AmpliSens-1 RG, AmpliSens-1 iQ, AmpliSens-1 Mx general programs allows simultaneous conducting of any combination of tests (for instance, for detection of pathogens of sexually transmitted diseases) by the same amplification program. Analytical performances of AmpliSens® *HHV6*-screen-titre-FRT PCR kit are stable if general program is applied.

8. DATA ANALYSIS.

Accumulation of *HHV-6* DNA (C+) amplification product is detected on the **JOE /Yellow/HEX** channel, **β-globin gene (IC)** amplification product is detected on the **FAM /Green** channel.

The results are interpreted by the crossing (or not) of the fluorescence curve with the threshold line that corresponds with presence (or absence) of Ct value in the special column of the results grid.

See **Appendix 2-4** for data analysis settings.

The analysis results are considered valid, only if the control samples results comply the following:

Table 5

Results for controls

Control	Stage for control	Ct channel FAM (Green)	Ct channel JOE (Yellow)	Interpretation
C-	DNA isolation	Neg	Neg	OK
NCA	Amplification	Neg	Neg	OK
C+	Amplification	≤ X*	≤ X*	OK
HG1	Amplification	Pos	Pos	OK
HG2	Amplification	Pos	Pos	OK
HG3	Amplification	Pos	Pos	OK

*For X value see Appendices 2, 3, 4.

HHV6 DNA and Glob IC DNA values are used for calculation of *HHV6* DNA concentration in every sample. It is necessary to estimate DNA Glob concentration in advance: this value should not exceed 10⁴ DNA copies per reaction for blood and biopsy samples; and 10³ DNA copies per reaction for saliva samples, throat washes and swabs.

Calculation of concentration in logarithm of *HHV6* DNA copies per 10⁵ cells in control and clinical samples

$$\lg \left\{ \frac{\text{number_copies_DNA_HHV6_per_PCR-sample}}{\text{number_copies_DNA_HHV6_per_PCR-sample}} \times 2 \times 10^5 \right\} = \lg(\text{HHV-6 DNA copies}/10^5 \text{ cells})$$

Calculation of concentration in *HHV6* DNA copies per 10⁵ cells in control and clinical samples

$$x2 \times 10^5 \frac{\text{number_copies_DNA_HHV6_per_PCR-sample}}{\text{number_copies_DNA_HHV6_per_PCR-sample}} = \text{HHV-6 DNA copies}/10^5 \text{ cells}$$

9. TROUBLESHOOTING.

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

- If any Ct value appears in JOE/HEX channel for Negative Controls (C-) or in any channel for Negative Control (NCA), it indicates the contamination of reagents or samples. In this case results of the analysis for all samples are considered invalid. It is necessary to repeat the analysis of all tests, and also to take measures to detect and eliminate the source of contamination.
- Positive Control results should fit in a range specified in Important product information bulletin to AmpliSens® *HHV6*-screen-titre-FRT PCR kit, otherwise the analysis for all samples should be repeated.
- If the sample's Ct value on FAM/Green channel is absent or more than needed, then the errors has been made during extraction. The test must be repeated from extraction stage.
- If the calculated concentration of standard samples differs from the given concentration more than 30%, a mistake in tubes placing has probably occurred. Check the tubs order in the instrument.
- If the correlation coefficient, R, in the **Standard Curve** window, less than 0.98 please check the correctness of calibrators. If it doesn't work, perform PCR again.

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

Reproducibility of quantitative analysis results:

Variation coefficient of *HHV6* DNA/10⁵ cells concentrations values for Positive Control should not exceed 25 % in a single run and 35 % in different runs.

10. STABILITY AND STORAGE.

All components of the **AmpliSens® *HHV6*-screen-titre-FRT PCR kit** (except for Polymerase(TaqF), PCR-mix-2-FRT, and PCR-mix-1-FRT *HHV-6*/Glob) are to be stored between 2 and 8 °C. All components of the **AmpliSens® *HHV6*-screen-titre-FRT PCR kit** are to be stable until the expiry date stated on the label.



Polymerase (TaqF), PCR-mix-2-FRT, and PCR-mix-1-FRT *HHV-6*/Glob are to be stored at not more than minus 16°C.

11. SPECIFICATIONS.

11.1. Sensitivity.

Analytical sensitivity of **AmpliSens® HHV6-screen-titre-FRT** PCR kit is no less than 1×10^3 genome equivalents of DNA *HHV6* per 1ml of a sample (GE/ml).



The claimed analytical features of **AmpliSens® HHV6-screen-titre-FRT** PCR kit are guaranteed only when additional reagent kits (see Table 1 of this manual) are used.

11.2. Specificity.

Specificity of **AmpliSens® HHV6-screen-titre-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® HHV6-screen-titre-FRT** PCR kit was confirmed in laboratory clinical tests.

12. REFERENCES.

1. 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 compliance with Federal State Institution of Science "Central Research Institute of Epidemiology" ISO 13485 – certified Quality Management System, each lot of **AmpliSens® HHV6-screen-titre-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

14. EXPLANATION OF SYMBOLS.



Manufacturer



Use by



For *in Vitro* Diagnostic Use



Catalogue number



Contains sufficient for <n> tests



Consult instructions for use



For working with Rotor-Gene™ 3000/6000



Positive control



Temperature limitation



Batch code



Version



Internal Control complex



Authorized representative in the European Community.



Caution, consult accompanying documents



For working with iQ5, iQ, iCycler



Negative control