# AmpliSens® *HHV6*-screen-titre-FRT
PCR kit

For Professional Use Only

# Instruction Manual

## KEY TO SYMBOLS USED

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

## 1. INTENDED USE

**AmpliSens® *HHV*6-screen-titre-FRT** PCR kit is an *in vitro* nucleic acid amplification test for qualitative and quantitative detection of *human herpes virus* type 6 (*HHV*6) DNA in the clinical material (whole human blood, white blood cells, viscera biopsy material, saliva, oropharyngeal swabs and cerebrospinal fluid (liquor)) 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

*HHV*6 DNA detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific *HHV*6 primers. In the real-time PCR, the amplified product is detected with the use of fluorescent dyes. These dyes are linked to oligonucleotide probes, which bind specifically to the amplified product during thermocycling. The real-time monitoring of fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run.

**AmpliSens® *HHV*6-screen-titre-FRT** PCR kit is a qualitative test based on the use of an endogenous control, the β-globin gene. The DNA target selected as an endogenous internal control is a human genome fragment. The use of an endogenous internal control makes it possible not only to monitor test stages (DNA extraction and amplification) but also to assess the adequacy of sampling and storage of clinical material.

**AmpliSens® *HHV*6-screen-titre-FRT** PCR kit uses "hot-start," which greatly reduces the frequency of nonspecifically primed reactions. "Hot-start" is guaranteed by using a 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 dUTP. 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 dUTP 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 |
|---|---|---|
| DNA-target | DNA (IC) Glob | DNA *Human betaherpesvirus 6A/B* |
| Target gene | DNA fragment of the b-globin gene | DNA fragment of the DNA polymerase catalytic subunit protein (U38 gene) |

## 3. CONTENT

**AmpliSens® *HHV*6-screen-titre-FRT PCR** kit is produced in 1 form:
**variant FRT-100 F,** REF R-V10-T(RG,iQ,Mx)-CE.

**Variant FRT-100 F** includes:

| Reagent | | Description | Volume, ml | Quantity |
|---|---|---|---|---|
| **PCR-mix-1-FRT *HHV*-6 / Glob** | | clear liquid from colorless to light lilac colour | 0.6 | 2 tubes |
| **PCR-mix-2-FRT** | | colorless clear liquid | 0.3 | 2 tubes |
| **Polymerase (TaqF)** | | colorless clear liquid | 0.03 | 2 tubes |
| **RNA-buffer** | | colorless clear liquid | 0.6 | 1 tube |
| **DNA calibrators** | **KSG1** | colorless clear liquid | 0.2 | 1 tube |
| | **KSG2** | colorless clear liquid | 0.2 | 1 tube |
| **Negative Control (C–)*** | | colorless clear liquid | 1.2 | 2 tubes |
| **Positive Control DNA *HHV*-6 and human DNA**** | | colorless clear liquid | 0.2 | 2 tubes |

* must be used in the extraction procedure as Negative Control of Extraction (C–).
** must be used in the extraction procedure as Positive Control of Extraction (PCE).
**Variant FRT-100 F** is intended for 110 reactions (including controls).

## 4. ADDITIONAL REQUIREMENTS

- Hemolytic.
- DNA extraction kit.
- Disposable powder-free gloves and a laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol filters (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with a rotor for 2-ml reaction tubes.
- PCR box.
- Real-time instruments (for example, Rotor-Gene 3000/6000 (Corbett Research, Australia); Rotor-Gene Q (QIAGEN, Germany), iCycler iQ or iQ5 (Bio-Rad, USA), Mx3000P (Stratagene, USA), or equivalent).
- Disposable polypropylene PCR tubes (0.1- or 0.2-ml):
  a) 0.2-ml PCR tubes with domed caps if a plate-type instrument is used;
  b) 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 at the temperature from 2 to 8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir 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 a 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 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.
- Samples should be considered potentially infectious and handled in biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all samples or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
- Avoid 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 immediately.
- Safety Data Sheets (SDS) are available on request.
- 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 where 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 are described in the manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

**AmpliSens® *HHV*6-screen-titre-FRT** PCR kit is intended for the analysis of DNA extracted with DNA extraction kits from the clinical material (whole human blood, white blood cells, viscera biopsy material, saliva, oropharyngeal swabs, and cerebrospinal fluid (liquor)).

*Whole peripheral and umbilical blood*

Before extraction, it is necessary to pretreat blood. Add 1.0 ml of **Hemolytic** (REF 137-CE, manufactured by Federal Budget Institute of Science "Central Research Institute for Epidemiology") and 0.25 ml of whole blood to 1.5-ml Eppendorf tube using an individual tip. Carefully vortex the contents of the tube and incubate it for 10 min with periodic stirring. Centrifuge tubes at 8,000 rpm for 2 min. Remove the supernatant using vacuum aspirator. Do not disturb the pellet. After washing, the pellet should be white. A small quantity of a pinkish film above the pellet (erythrocyte debris) is allowed. Washing with Hemolytic can be repeated, if necessary. Thus obtained leukocyte pellet should be lysed immediately (in case of RIBO-prep extraction, add 300 µl of Solution for Lysis and then extract DNA according to the RIBO-prep instruction manual; do not add Solution for Lysis again) or it can be stored at ≤ 68 °C for a long time.

*Packed white cells of peripheral and/or umbilical blood*

It is obtained from peripheral and/or umbilical blood. Blood can be stored for 6 hours after sampling at room temperature. To obtain white cells, centrifuge tube with blood at 800-1,600 g (3,000 rpm) for 20 min. Then, collect the white film formed on the surface of the supernatant and carry out the pretreatment as described for whole peripheral and umbilical blood. White cells of peripheral and umbilical cord blood can be stored at ≤ –68 °C for a long time.

## 7. WORKING CONDITIONS

**AmpliSens® *HHV*6-screen-titre-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:

- **RIBO-prep**, REF K2-9-Et-100-CE,
- **DNA-sorb-B**, REF K1-2-100-CE.
- **DNA-sorb-C**, REF K1-6-100-CE (for viscera biopsy material).

In the extraction procedure it is necessary to carry out the control reaction:

**C–** – Add **100 µl of Negative Control (C–)** to the tube labelled C– (Negative Control of Extraction).

**PCE:** – Add **90 µl** of **Negative Control (C–)** and **10 µl** of **Positive Control DNA *HHV*-6 and human DNA** to the tube labeled **PCE** (Positive Control of Extraction)**.**

**NOTE:** The results of PCR analysis are taken into account in complex diagnostics of disease.

### 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**.
The type of tubes depends on the type of PCR real-time instrument.
Use disposable filter tips for adding reagents, cDNA and control samples into tubes.

1. Prepare the mixture of **PCR-mix-2-FRT** and **Polymerase (TaqF)**. For this purpose transfer the content of one tube with **Polymerase (TaqF) (30 µl)** into the tube with **PCR-mix-2-FRT (300 µl).** Vortex carefully avoiding foaming. Mark the tube with the mixture preparation date.

**NOTE:** The prepared mixture is intended for analysis of 60 samples including controls. The mixture can be stored at the temperature 2-8 °C for 3 months and used as needed.

**NOTE:** If the mixture cannot be used up for 3 months, prepare the mixture for a smaller number of reactions. For example, mix **150 µl of PCR-mix-2-FRT** and **15 µl of polymerase (TaqF).** Thus obtained mixture is intended for 30 reactions.

2. Prepare the reaction mixture. Note that, for analysis of even **one** DNA sample in **the qualitative format**, it is necessary to run **two controls** of amplification: the Positive Control of Amplification (**KSG2**) and the Negative Control of Amplification (**RNA-buffer**). For analysis of even **one** DNA sample in the **quantitative format**, it is necessary to run **five controls** of amplification: two DNA calibrators (**KSG1** and **KSG2**) in two replicates and the Negative Control of Amplification (**RNA-buffer**). In addition, take reagents for one extra reaction.
3. Mix **PCR-mix-1-FRT *HHV*-6 / Glob** and the mixture of **PCR-mix-2-FRT** and **polymerase (TaqF)** prepared earlier in an individual tube in the following proportion:
   – **10 µl of PCR-mix-1-FRT *HHV*-6 / Glob,**
   – **5 µl of PCR-mix-2-FRT and polymerase (TaqF)** mixture.
   Calculate the required number of reactions including the test and control samples (see Table 2).

Table 2

**Scheme of reaction mixture preparation**

| **Total reaction volume is 25 µl including the volume of DNA sample – 10 µl.** | | | |
|---|---|---|---|
| **Reagent volume per 1 reaction, µl** | | **10,0** | **5,0** |
| **Number of clinical samples** | | **PCR-mix-1-FRT *HHV-6* / Glob**[1] | **Mixture of PCR-mix-2-FRT and polymerase (TaqF)**[1] |
| **For quantitative analysis** | **For qualitative analysis** | | |
| **1** | **4** | **70** | **35** |
| **2** | **5** | **80** | **40** |
| **3** | **6** | **90** | **45** |
| **4** | **7** | **100** | **50** |
| **5** | **8** | **110** | **55** |
| **6** | **9** | **120** | **60** |
| **7** | **10** | **130** | **65** |
| **8** | **11** | **140** | **70** |
| **9** | **12** | **150** | **75** |
| **10** | **13** | **160** | **80** |
| **11** | **14** | **170** | **85** |
| **12** | **15** | **180** | **90** |
| **13** | **16** | **190** | **95** |
| **14** | **17** | **200** | **100** |
| **15** | **18** | **210** | **105** |
| **16** | **19** | **220** | **110** |
| **17** | **20** | **230** | **115** |
| **18** | **21** | **240** | **120** |
| **19** | **22** | **250** | **125** |
| **20** | **23** | **260** | **130** |
| **21** | **24** | **270** | **135** |
| **22** | **25** | **280** | **140** |
| **23** | **26** | **290** | **145** |
| **24** | **27** | **300** | **150** |
| **25** | **28** | **310** | **155** |
| **30** | **33** | **360** | **180** |

**NOTE:** If 60 samples are analyzed simultaneously, a simplified scheme of mixture preparation can be used. Transfer the content of one tube with **PCR-mix-2-FRT** and the content of one tube with **polymerase (TaqF)** into the tube with **PCR-mix-1-FRT *HHV*-6 / Glob**.

4. Take the required number of tubes for amplification of test and control DNA samples. Transfer 15 µl of the prepared mixture to each tube.
5. Add 10 µl of DNA samples obtained at the DNA extraction stage into each tube with the reaction mixture.
6. <u>For qualitative analysis:</u>

**NCA** – Add **10 µl** of **RNA-buffer** to the tube labeled NCA (Negative Control of Amplification).

**C+** – Add **10 µl** of **KSG2** to the tube labeled C+ (Positive Control of Amplification).

**C–** – Add **10 µl** of **the sample extracted from the Negative Control (C–) reagent** to the tube labeled C– (Negative control of Extraction).

**PCE** – Add **10 µl** of **the sample extracted from the Positive control DNA *HHV*-6 and human DNA reagent** to the tube labeled PCE (Positive control of

<u>For quantitative analysis:</u>

**NCA** – Add **10 µl** of **RNA-buffer** to the tube labeled NCA (Negative Control of Amplification).

**DNA calibrators KSG1 and KSG2** – Add **10 µl** of **KSG1** to two tubes and add **10 µl** of **KSG2** to other two tubes.

**C–** – Add **10 µl** of **the sample extracted from the Negative Control (C–) reagent** to the tube labeled C– (Negative control of Extraction).

**PCE** – Add **10 µl** of **the sample extracted from the Positive control DNA *HHV*-6 and human DNA reagent** to the tube labeled PCE (Positive control of Extraction).

#### 8.2.2. Amplification

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

Table 3

**AmpliSens-1 amplification program**

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

Fluorescent signal is detected in the channels for the FAM and JOE fluorophores.

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

[1] Values are given with account of one extra reaction and five controls (2 DNA calibrators KSG1 and KSG2 (in two replicates), negative control (RNA-buffer) for quantitative analysis of *HHV*6 DNA, and two controls (positive and negative) for qualitative analysis of *HHV*6 DNA.
[2] For example, Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q or equivalent.
[3] For example, iCycler iQ5, Mx3000P, Mx3000 or equivalent.

## 9. DATA ANALYSIS

Analysis of results is performed by the software of the real-time PCR instrument used by measuring fluorescence signal accumulation in two channels:

– The signal of **β-globin gene DNA (IC Glob)** amplification product is detected in the channel for the FAM fluorophore.
– The signal of the ***HHV6* DNA (Positive Control DNA *HHV*-6 and human DNA)** amplification product is detected in the channel for the JOE 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:

– *HHV*6 DNA is **detected** if the *Ct* value determined in the results grid in the channel for the JOE fluorophore does not exceed the boundary value. Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.
– *HHV*6 DNA is **not detected** if the *Ct* value is not determined (absent) in the results grid in the channel for the JOE fluorophore (the fluorescence curve does not cross the threshold line), whereas for qualitative analysis the *Ct* value in the channel for the FAM fluorophore does not exceed the boundary *Ct* value specified in the *Important Product Information Bulletin* and for quantitative analysis the quantity of IC Glob DNA is greater than 2000 copies per reaction in the case of whole blood, white blood cells, and viscera biopsy material and greater than 500 copies per reaction in the case of saliva and oropharyngeal swabs. For cerebrospinal fluid (liquor), the *Ct* value in the results grid in the channel for the FAM fluorophore can be greater than the boundary *Ct* value specified in *the Important Product Information Bulletin* or the quantity of IC Glob DNA can be less than 500 copies per reaction in the case of quantitative analysis because cerebrospinal fluid samples may contain a very small number of cells.
– The result of analysis is **invalid** if the *Ct* value is not determined (absent) in the results grid or if it is greater than the boundary *Ct* value in the channel for the JOE fluorophore whereas for qualitative analysis the *Ct* value in the channel for the FAM fluorophore exceeds the *Ct* value specified in the *Important Product Information Bulletin* and for quantitative analysis the quantity of IC Glob DNA is less than 2000 copies per reaction for whole blood, white blood cells, viscera biopsy material and less than 500 copies per reaction for saliva, oropharyngeal swabs. In such case the PCR analysis should be repeated for required sample.
– The result of analysis is **equivocal** if the *Ct* value in the channel for the JOE fluorophore exceeds the boundary *Ct* value specified in the *Important Product Information Bulletin.* It is necessary to carry out the additional analysis for such sample with two repeats. If a reproducible positive result is obtained, the result is considered positive. If the obtained *Ct* values are not reproducible in two repeats, the result is considered **equivocal.**
– For qualitative analysis, the negative result is considered **unreliable** if the *Ct* value in the channel for the FAM fluorophore exceeds the boundary *Ct* value specified in the *Important Product Information Bulletin.* For quantitative analysis, the quantitative positive or negative result is considered **unreliable** if the quantity of IC Glob DNA is less than 2000 copies per reaction in the case of whole blood, white blood cells, and viscera biopsy material or less than 500 copies per reaction in the case of saliva and oropharyngeal swabs

**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 controls C–, PCE, NCA, C+, KSG1, and KSG2 are correct (see Table 3). For quantitative analysis the results for Positive Control should fall in the concentration range specified in the *Important Product Information Bulletin.***

Table 4

**Results for controls**

| Control | Stage for control | Amplification results in the channel for the fluorophore | | | |
|---|---|---|---|---|---|
| | | FAM | | JOE | |
| | | Qualitative format | Quantitative format | Qualitative format | Quantitative format |
| **C–** | DNA extraction, PCR | Absent | Absent | Absent | Absent |
| **PCE** | DNA extraction, PCR | *Ct* < boundary value | *Ct* < boundary value | *Ct* < boundary value | concentration value falls in the range specified in the *Important Product Information Bulletin* |
| **NCA** | PCR | Absent | Absent | Absent | Absent |
| **C+** | PCR | *Ct* < boundary value | – | *Ct* < boundary value | – |
| **KSG1, KSG2** | PCR | – | *Ct* value and calculated concentration are defined | – | *Ct* value and calculated concentration are defined |

**For whole human blood, white blood cells, or viscera biopsy material calculate the concentration in logarithm of *HHV*6 DNA copies per the standard cell quantity ($10^5$) in control and test samples using the following formula:**

$$\lg\left\{\frac{number\ of\ HHV6\ DNA\ copies\ in\ PCR\ sample}{number\ of\ Glob\ DNA\ copies\ in\ PCR\ sample}\cdot\times 2\times 10^5\right\}=\lg\{HHV6\ DNA\ copies/10^5 5\ cells\}$$

**For saliva, oropharyngeal swabs, and cerebrospinal fluid (liquor) calculate the concentration of *HHV*6 DNA per ml of sample (CS *HHV*6 DNA) using the following formula:**

**CS*HHV*6 DNA = K*HHV*6 DNA x 100 (copies/ml)**

**K *HHV*6 DNA is the quantity of *HHV*6 DNA copies in the DNA sample.**

## 10. TROUBLESHOOTING

Results of analysis are not taken into account in the following cases:

1. If any *Ct* value appears in the results grid in the channels for the FAM and JOE fluorophores for Negative Control of Amplification (NCA) and Negative Control of Extraction (C–), it indicates contamination of reagents or samples. Repeat PCR analysis for all samples in which *HHV*6 DNA was detected starting from the DNA extraction stage.
2. In qualitative analysis, if the *Ct* value for the Positive Control of Amplification (C+) – **KGS2** – in the channel for the JOE (*HHV*6) or FAM fluorophore is absent or greater than the boundary value in the results grid, repeat amplification of all samples in which *HHV6* DNA was not detected.
3. If the *Ct* value for the Positive Control of Extraction (PCE) – **Positive Control DNA *HHV*-6 and human DNA**) – in the channel for the JOE (*HHV*6) or FAM fluorophore is absent or greater than the boundary value, the results of analysis of all samples are considered invalid. Repeat analysis of all samples starting from the DNA amplification stage.
4. If the *Ct* value for a sample in the channel for the JOE fluorophore is absent or exceeds the boundary *Ct* value and the *Ct* value for the same sample in the channel for the FAM fluorophore exceeds the boundary *Ct* value specified for Internal Control, repeat analysis of the sample starting from the DNA extraction stage. This may be caused by errors in preparation of clinical material, which entailed the loss of DNA, or by the presence of inhibitors.
5. If the *Ct* value for a clinical sample in the channel for the JOE fluorophore (*HHV*6 DNA) exceeds the boundary *Ct* value, the result of analysis is considered **equivocal**. Repeat analysis of such samples in duplicate. If a positive result is obtained in both replicates, the result of analysis is considered as **positive**. If the result is not reproduced in two replicates, the result of analysis of such samples is considered **equivocal**.
6. If the number of copies per reaction in DNA calibrators in quantitative tests exceeds the specified value by more than 30%, check the order of the tubes in the rotor (DNA calibrators should be inserted into the cells named ***Standard*** in the table of samples, the concentration of samples should correspond to the concentration specified in the *Important Product Information Bulletin*, and cell no. 1 in rotor-type instruments should not be empty (fill it with any test tube).
7. If the correlation coefficient **R** in the ***Standard Curve*** window in quantitative tests is less than 0.9, this indicates error in calibration. Check whether the settings for DNA calibrators are correct and change them, if necessary. If this does not help, run PCR for all samples and calibrators.

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

## 11. TRANSPORTATION

**AmpliSens® *HHV*6-screen-titre-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®** ***HHV*6-screen-titre-FRT** PCR kit (except for PCR-mix-1-FRT *HHV*-6 / Glob, polymerase (TaqF) and PCR-mix-2-FRT) are to be stored at 2–8 °C when not in use. All components of the **AmpliSens®** ***HHV*6-screen-titre-FRT** PCR kit are stable until the expiration date on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

**NOTE:** PCR-mix-1-FRT *HHV*-6 / Glob, polymerase (TaqF), and PCR-mix-2-FRT are to be stored at temperature from minus 24 to minus 16 ºC when not in use.

**NOTE:** PCR-mix-1-FRT *HHV*-6 / Glob should be kept away from light.

## 13. SPECIFICATIONS

### 13.1. Sensitivity

The analytical sensitivity of **AmpliSens®** ***HHV6*-screen-titre-FRT** PCR kit is the following:

| Clinical material | Nucleic acid extraction kit | Sensitivity |
|---|---|---|
| Cerebrospinal fluid (liquor), saliva, oropharyngeal swabs | RIBO-prep | 400 copies per 1 ml |
| Whole human blood, white blood cells and viscera biopsy material | RIBO-prep | 5 copies per $10^5$ cells |

Linear range of **AmpliSens®** ***HHV*6-screen-titre-FRT** PCR kit is **500–10.000.000 copies/ml**

### 13.2. Specificity

The analytical specificity of **AmpliSens®** ***HHV*6-screen-titre-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.

Nonspecific reactions were absent is tests with DNA of other viruses (herpes simplex virus types 1 and 2, Epstein-Barr human virus, human herpes virus type 8, varicella-zoster virus, parvovirus B19, and others), bacterial pathogens (*Staphylococcus aureus, Streptococcus pyogenes, Streptococcus agalactiae,* and others) and human DNA.

The clinical specificity of **AmpliSens®** ***HHV*6-screen-titre-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 State Institute of Science “Central Research Institute of Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being.
2. Guidelines to the **AmpliSens®** ***HHV*6-screen-titre-FRT** PCR kit for qualitative and quantitative detection of *human herpes virus* type 6 (*HHV*6) DNA in clinical materials by the polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection, developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology”.


## 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 **AmpliSens®** ***HHV*6-screen-titre-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 |
|---|---|---|
| 07.12.11 VV | Specifications, Sensitivity | The linear measurement range was added |
| 27.04.15 ME | Through the text | Corrections according to the template. Grammar corrections |
| | 8.2.1 Preparing tubes for PCR | Appendix 1 was integrated into the text of the instruction manual as Table 1 |
| | 9. Data analysis | The sections were rewritten |
| | 10. Troubleshooting | |
| 21.12.18 TA | 2. Principle of PCR detection | The information about the enzyme UDG was added. The information about "hot-start" was corrected |
| 13.02.19 DV | 3. Content | The color of the reagent was specified |
| 13.07.20 KK | Through the text | The text formatting was changed |
| | 2. Principle of PCR detection | The table with targets was added |
| | Footer | The phrase "Not for use in the Russian Federation" was added |
| 19.01.21 EM | 10. Troubleshooting | The information for Negative Control of Amplification (NCA) and Negative Control of Extraction (C–) was corrected |
| 23.03.21 EM | — | The name, address and contact information for Authorized representative in the European Community was changed |

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