



For Professional Use Only

eSens HHV6 QT PCR kit

REF ES3206A

Instructions for Use

1 INTENDED USE

eSens HHV6 QT PCR kit is an *in vitro* nucleic acid amplification test for qualitative and quantitative detection of *human herpes virus type 6* (HHV6) 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

HHV6 DNA detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific HHV6 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.

eSens HHV6 QT PCR kit includes an endogenous control, the β -globin gene detection. 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.

eSens HHV6 QT 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 <i>Human betaherpesvirus 6A/B</i>
Target gene	DNA fragment of the b-globin gene	DNA fragment of the DNA polymerase catalytic subunit protein (U38 gene)

3 CONTENT

eSens HHV6 QT PCR kit (ES3206A) contains:

Reagent	Description	Volume, ml	Quantity	
PCR-mix-1-FRT <i>HHV-6</i> / 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 <i>HHV-6</i> 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).

eSens HHV6 QT PCR kit is intended for 110 reactions (including controls).

4 ADDITIONAL REQUIREMENTS

- 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.
- Desktop centrifuge with a rotor for 2-ml reaction tubes.
- PCR box.
- Real-time instruments (for example, Rotor-Gene Q (QIAGEN, Germany), CFX 96 Touch, CFX 96 Opus (Bio-Rad, USA), QuantStudio 5 (Thermo Fisher Scientific), or equivalent).
- Disposable polypropylene PCR tubes (0.1- or 0.2-ml):
 - 0.2-ml PCR tubes with domed caps if a plate-type instrument is used;
 - 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.

6 SAMPLING AND HANDLING

eSens HHV6 QT 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 blood: collected in ACD or EDTA tubes

Sputum: add 1 volume of sputum to 1 volumes of saline water and vortex vigorously. Centrifuge at 10000g/min for 10 min. Discard the supernatant and leave about 100 µl of solution for DNA extraction.

Swabs: insert the swab into the nuclease-free 1,5 ml tube and add 0,2 ml of Transport medium. Vigorously agitate swabs in medium for 15-20 sec.

Packed white cells of 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.

7 WORKING CONDITIONS

eSens HHV6 QT PCR kit should be used at 18–25 °C

8 PROTOCOL

8.1 DNA extraction

Any commercial nucleic acid extraction kit, if IVD-CE validated for the indicated specimen types, could be used.

Ecoli Dx, s.r.o. recommends:

- For the manual extraction
 - **RIBO-prep** (K2-9-Et-100-CE)
 - **DNA-sorb-B** (K1-2-100-CE)
- For the automatic extraction
 - **ePure Viral Nucleic acid Extraction Kit** (E2003)
 - **ePure Blood DNA Extraction Kit** (E2001)

Please carry out nucleic acid extraction according to the manufacture´s instruction.

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 <i>HHV-6</i> / Glob*	Mixture of PCR-mix-2- FRT and polymerase (TaqF)*
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

* 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 HHV6 DNA, and two controls (positive and negative) for qualitative analysis of HHV6 DNA.

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. For qualitative analysis:

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 Extraction)

For quantitative analysis:

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

eSens-1 amplification program

Step	Rotor-type Instruments (for example, Rotor-Gene Q or equivalent)			Plate-type Instruments (for example, CFX 96 Touch, CFX 96 Opus, QuantStudio 5 or equivalent)		
	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 <i>Fluorescence acquiring</i>		60	30 s <i>Fluorescence acquiring</i>	
	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 Technical Sheet.
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.

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:

- **HHV6 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.

- *HHV6* 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 Technical Sheet 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 Technical Sheet 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 Technical Sheet 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 Technical Sheet. 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 Technical Sheet. 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 Technical Sheet enclosed to the PCR kit.

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 4). For quantitative analysis the results for Positive Control should fall in the concentration range specified in the Technical Sheet.

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 <i>Technical Sheet</i>
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 HHV6 DNA copies per the standard cell quantity (10^5) in control and test samples using the following formula:

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

For saliva, oropharyngeal swabs, and cerebrospinal fluid (liquor) calculate the concentration of HHV6 DNA per ml of sample (CS HHV6 DNA) using the following formula:

$$\text{CSHHV6 DNA} = \text{KHHV6 DNA} \times 100 \text{ (copies/ml)}$$

K HHV6 DNA is the quantity of HHV6 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 HHV6 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 (HHV6) 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.

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 (HHV6) 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.

3. 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.
4. If the *Ct* value for a clinical sample in the channel for the JOE fluorophore (HHV6 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**.
5. 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 *Technical Sheet*, and cell no. 1 in rotor-type instruments should not be empty (fill it with any test tube).
6. 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.

11 TRANSPORTATION

eSens HHV6 QT PCR kit should be transported at 2–8 °C for no longer than 5 days.

12 STABILITY AND STORAGE

All components of the **eSens HHV6 QT 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 **eSens HHV6 QT 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 °C 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 **eSens HHV6 QT PCR kit** is the following:

Clinical material	Nucleic acid extraction kit	Sensitivity
Cerebrospinal fluid (liquor), saliva, oropharyngeal swabs	RIBO-prep ePure Viral Nucleic acid Extraction Kit	400 copies per 1 ml
Whole human blood, white blood cells and viscera biopsy material	RIBO-prep ePure Blood DNA Extraction Kit	5 copies per 10 ⁵ cells

Linear range of **eSens HHV6 QT PCR kit** is **500–10.000.000 copies/ml**

13.2 Specificity

The analytical specificity of **eSens HHV6 QT 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 in 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 **eSens HHV6 QT 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 **eSens HHV6 QT PCR kit** for qualitative and quantitative detection of *human herpes virus type 6 (HHV6)* 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

The production process, including batch release, is carried out in accordance with an established quality management system certified according to ISO 13485.

16 KEY TO SYMBOLS USED

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

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
01_04/2022		

Ecoli Dx, s.r.o. , Purkyňova 74/2



110 00 Praha 1, Česká republika
Tel: +420 325 209 912

Mobil: +420 739 802 523