ECOLI

eSens

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IVD

For Professional Use Only

eSens HHV7 QT PCR kit

REF ES3207A

Instructions for Use

1 INTENDED USE

eSens HHV7 QT PCR kit is an *in vitro* nucleic acid amplification test for quantitative detection of *human herpes virus* type 7 (*Human betaherpesvirus* 7, *HHV7*) DNA in the biological material (blood plasma, whole blood, saliva, oropharyngeal swab, cerebrospinal fluid), using real-time hybridization-fluorescence detection of amplified products. The material for PCR is DNA samples extracted from test material.

Indications and contra-indications for use of the reagent kit

The reagent kit is used to study biological material obtained from persons with suspected infection caused by *HHV7*, regardless of the form and presence of the disease manifestation. There are no contra-indications with the exception of cases when the material cannot be taken for medical reasons.

Potential users of a medical device

Only medical workers trained in the methods of molecular diagnostics and the rules of work in the clinical diagnostic laboratory in the prescribed manner (SP 1.3.2322-08 "Safety of work with microorganisms of III-IV groups of pathogenicity (danger) and causative agents of parasitic diseases").

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

2 PRINCIPLE OF PCR DETECTION

The principle of testing is based on the DNA extraction from test samples together with the exogenous internal control (Internal Control-FL (IC)) and simultaneous amplification of DNA fragments of the detected microorganism and DNA of the exogenous and endogenous internal control with hybridization-fluorescence detection.

DNA extraction is carried out in the presence of the exogenous internal control (Internal Control-FL (IC)) in order to control all PCR-analysis stages of each individual sample and to identify possible reaction inhibition. The DNA fragment of the human β-globin gene (IC Glob) is used as an endogenous internal control and allows not only to control all stages of PCR study, but also to evaluate the adequacy of material collection, transportation and storage. Being a part of the human genome, IC Glob DNA must always present in biological material containing human cells (whole blood and oropharyngeal swab).

Amplification of a DNA fragments with the use of specific primers and Taq-polymerase enzyme are performed with the DNA/RNA samples obtained at the extraction stage. 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.
The quantitative analysis of *HHV*7 DNA is based on the linear dependence between the initial concentration logarithm of DNA target in a test sample and the cycle threshold (*Ct*) (the cycle of beginning of fluorescence signal exponential growth). For the quantitative analysis amplification of DNA from the test samples is carried out simultaneously with DNA- calibrators (samples with the known concentration of the DNA target). Based on the amplification results of DNA-calibrators a calibration line is plotted and it is used for the estimation of concentration of the DNA target in the test samples.
The PCR kit contains the system for prevention of contamination by amplicons using the enzyme uracil-DNA-glycosylase (UDG) and deoxyuridine triphosphate (dUTP).
The results of amplification are registered in the following fluorescence channels (Table 1):

Table 1

Channel for fluorophore	**FAM**	**JOE**	**ROX**
DNA-target	fragment of human DNA (IC Glob)	*HHV7* DNA	Internal Control-FL (IC) DNA
Target gene	β-globin gene	*MCP*-gene	artificially synthesized sequence

3 CONTENT

eSens HHV7 QT PCR kit (ES3207A) includes:

Reagent	**Description**	**Volume, ml**	**Quantity**
PCR-mix-FL *HHV7*	clear liquid from colorless to light lilac colour	1.2	1 tube
PCR-buffer-H	colorless clear liquid	0.6	1 tube
C1 *HHV7*	colorless clear liquid	0.2	1 tube
C2 *HHV7*	colorless clear liquid	0.2	1 tube
TE-buffer	colorless clear liquid	0.2	1 tube
Internal Control-FL (IC)*	colorless clear liquid	1.0	1 tube
Negative Control (C–)**	colorless clear liquid	1.2	2 tubes
Positive Control *HHV7****	colorless clear liquid	0.1	1 tube

***** add **10 µl** of **Internal Control-FL (IC)** during the DNA extraction procedure directly to the sample/lysis mixture.

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

******* must be used in the extraction procedure as Positive Control of Extraction.

eSens HHV7 QT PCR kit is intended for 110 reactions (including controls).

4 ADDITIONAL REQUIREMENTS

For sampling and pretreatment

- Transport medium for storage and transportation of respiratory swabs.
- Flocked-swab for collection, transportation and storage of biological samples.
- Plastic container (50-60 ml) for storage and transportation of biological samples.
- Vacuum tubes for sampling, storage and transportation of blood samples.
- Sterile bilateral needles for vacuum tubes intended for venous blood collection.
- Vacuette blood collection system.
- Medical centrifuge with equipment.
- Reagent for pretreatment of whole peripheral and umbilical blood.
- Microcentrifuge for Eppendorf tubes (RCF max. 12,000 x g).
- Vortex mixer.
- Vacuum aspirator with flask for removing supernatant.
- Pipettes (adjustable).
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir to throw off and inactivate the material.
- Disposable powder-free gloves and a laboratory coat.

For DNA extraction, reverse transcription and amplification

- DNA extraction kit.
- Disposable polypropylene PCR tubes:
  a) screwed or tightly closed 1.5-ml tubes for reaction mixture preparation.
  b) thin-walled 0.2-ml PCR tubes with optical transparent domed or flat caps or strips of eight 0.2-ml tubes with optical transparent caps if a plate-type instrument is used;
  c) thin-walled 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.
- Sterile pipette tips with filters (up to 100, 200 and 1,000 µl).
- Tube racks.
- PCR box.
- Vortex mixer.
- Pipettes (adjustable).
- 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)).
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir for used tips.
- Disposable powder-free gloves and a laboratory coat.

5 GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol filters 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 the PCR kit if the internal packaging was damaged or its appearance was changed.
- Do not use the PCR kit if the transportation and storage conditions according to the Instruction Manual were not observed.
- 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.
- The PCR kit is intended for single use for PCR analysis of specified number of samples (see the section “Content”).
- The PCR kit is ready for use in accordance with the Instruction Manual. Use the PCR kit strictly for intended purpose.
- 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 HHV7 QT PCR kit is intended for analysis of the DNA extracted with DNA extraction kits from the biological material (blood plasma, whole blood, saliva, oropharyngeal mucosa swab, cerebrospinal fluid).

Sampling

6.1 Blood plasma.

To obtain the plasma samples, blood should be taken after overnight fasting or in 3 hour after eating by a disposable 0.8-1.1 mm diameter needle into the tube with EDTA (special vacuum system **Vacuette** (lavender caps – 6 % EDTA)). After blood sampling the tube should be gently inverted several times for the thoroughly mixing with the anticoagulant. During 6 hours after blood sampling plasma should be transferred into a new tube. To do this the tubes with whole blood should be centrifuged at 3000 rpm for 10 min at room temperature. No less than 1 ml of obtained plasma is transferred by separate filter tips into sterile dry 2.0-ml tubes.
The samples can be stored before the pretreatment/PCR analysis:

- at the temperature from 2 to 8 °C – for 5 days,

- at the temperature from minus 24 to minus 16 °C – for 3 months,
- at the temperature from no more than minus 68 °C – for a long time.

Only one freeze-thaw cycle is allowed.

6.2 Whole blood.

Blood should be taken after overnight fasting or in 3 hour after eating by a disposable 0.8-1.1 mm diameter needle into the tube with EDTA (special vacuum system **Vacuette** (lavender caps – 6 % EDTA)). After blood sampling the tube should be gently inverted several times for the thoroughly mixing with the anticoagulant. The samples can be stored before the pretreatment / PCR research:

- at the temperature from 18 to 25 °C – for 2 h,
- at the temperature from 2 to 8 °C – for 72 h.

Do not freeze whole blood samples!

6.3 Saliva.

Saliva should be obtained after rinsing the oral cavity with water. Take saliva in sterile dry 2.0 ml tubes or in sterile plastic container (50-60 ml) in an amount not less than 0.5 ml. The samples can be stored before the pretreatment/ PCR research:

- at the temperature from 2 to 8 °C – for 24 h,
- at the temperature from minus 24 to minus 16 °C – for 3 months,
- at the temperature from no more than minus 68 °C – for a long time.

Only one freeze-thaw cycle is allowed.

6.4 Oropharyngeal swab.

Oropharyngeal swab is taken with a sterile dry probe with a viscose tip with rotating movements from the surface of the palatine arches and the posterior wall of the oropharynx. The probe tip is placed in a sterile disposable tube with 500 µL of transport medium for storage and transport of respiratory swabs. Carefully break off the polystyrene stick at a distance of no more than 0.5 cm from the working part and leave the working part of the probe with the biological instrument in the tube. Close the tube with solutions and the working area of the probe tip.
The samples can be stored before the pretreatment/PCR analysis:

- at the temperature from 2 to 8 °C – for 72 h,
- at the temperature from minus 24 to minus 16 °C – for 3 months,
- at the temperature from no more than minus 68 °C – for a long time.

Only one freeze-thaw cycle is allowed.

6.5 Cerebrospinal fluid.

Cerebrospinal fluid is taken by puncturing the lumbar, suboccipital region or cerebral ventricles with disposable puncture needles. The collection of cerebrospinal fluid in an amount of at least 1 ml is carried out in disposable sterile plastic tubes with a volume of at least 2 ml or containers.
The samples can be stored before the pretreatment/PCR analysis:

- at the temperature from 2 to 8 °C – for 24 h,
- at the temperature from minus 24 to minus 16 °C – for 3 months,
- at the temperature from no more than minus 68 °C – for a long time.

Only one freeze-thaw cycle is allowed.
It is allowed to transport samples of whole blood, blood plasma, oropharyngeal swab at a temperature of 2 to 8 °C for 72 hours, samples of saliva and cerebrospinal fluid at a temperature of 2 to 8 °C for 24 hours.

Pretreatment

6.6 Blood plasma and *saliva, oropharyngeal swab* and *cerebrospinal fluid*.

Pretreatment of blood plasma and saliva, oropharyngeal swab and cerebrospinal fluid samples is not required.

6.7 Whole blood samples.

Whole blood samples are to be prepared. Transfer 250 µl of whole blood to the disposable 1.5-ml tube. Add 1.0 ml of **Hemolytic** (137-CE) Gently vortex the tubes and leave them for 10 minutes at room temperature (from 18 to 25°C), stirring occasionally. Centrifuge at 8,000 rpm for 3 min. Remove the supernatant using vacuum aspirator leaving 100 µl of the pellet. After washing the cell pellet should be white, only a small pinkish bloom on the pellet is allowed (the remains of the destroyed erythrocytes). The washing using **Hemolytic** (137-CE) may be repeated if necessary. The obtained leucocytes pellet must be immediately lysed (in case of extraction using **RIBO-prep** (K2-9-Et-100-CE) add **300 µl of Solution for Lysis** and then extract DNA in accordance with the *Instruction Manual* enclosed to the **RIBO-prep** reagent kit without adding **Solution for Lysis** once again).
The whole blood samples prepared can be stored before the PCR:

- at the temperature from 2 to 8 °C – no more than 6 h,
- at the temperature from minus 24 to minus 16 °C – for 6 month.
- at the temperature from no more than minus 68 °C – for a long time.

Only one freeze-thaw cycle is allowed.

Interfering substances and limitations of using test material samples

The next samples are inapplicable for analysis:

- the whole blood samples, collected in the tubes with heparin as anticoagulant,
- the whole blood samples, containing blood clot or which has been exposed to freezing.

In order to control the DNA extraction efficiency and possible reaction inhibition the **Internal Control (Internal Control-FL (IC)**) is used in the PCR kit. The Internal Control is added in each biological sample at the extraction stage. The presence of internal control signal after the amplification testifies the effectiveness of nucleic acid extraction and the absence of PCR inhibitors.

Potential interfering substances

Endogenous and exogenous substances that may be present in the biological material (blood plasma, whole blood, saliva, oropharyngeal swab and cerebrospinal fluid) used for the study were selected to assess potential interference.
Samples without adding and with the addition of potentially endogenous and exogenous potential interfering substances were tested. The concentration of each potentially interfering substance is shown in Table 2. Samples of blood plasma, whole blood, saliva, oropharyngeal swab and cerebrospinal fluid with added quality control sample (QCS) containing *HHV7* DNA at concentration $1x10^5$ and $2x10^2$ copies/ml were tested.

Table 2

Type of tested material	Type of potential interferent	Potential interferent	Tested concentration in a sample	Interference presence
Blood plasma, whole blood	Endogenous substances	Total bilirubin	210 µmol/l (the upper limit of the norm is 21 µmol/l)	Not detected
		Total cholesterol	78 mmol/l (upper limit of normal - 7.8 mmol/l)	Not detected
		Triglycerides	37.0 mmol/l (upper limit of the norm - 3.7 mmol/l)	Not detected
		Hemoglobin	250 g/l (upper limit of the norm - 170 g/l)	Not detected
	Exogenous substances	Potassium EDTA	up to 2.0 mg/ml	Not detected
		Lithium heparin	from 12 IU/ml	Detected
Saliva, oropharyngeal swab	Exogenous substances	Chlorhexidine	0.5 %	Not detected
		Stomatofit	1.5 %	Not detected
		Miramistin	0.001 %	Not detected
Cerebrospinal fluid	Endogenous substances	Glucose	10 mmol/l (upper limit of normal - 3.89 mmol/l)	Not detected
		Leukocytes	500 cells/mm^3 (upper limit of the norm - 20 cells/mm^3)	Not detected

7 WORKING CONDITIONS

eSens HHV7 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), for DNA extraction from blood plasma, whole blood, saliva, oropharyngeal swabs and cerebrospinal fluid.

- For the **automatic** extraction

- **ePure Viral Nucleic Acid Extraction Kit** (E2003)

NOTE: Extract the DNA according to the manufacturer's protocol.
The DNA extraction for each sample is carried out in the presence of **Internal Control-FL (IC)**.

8.2 Preparing PCR

8.2.1 Preparing tubes for PCR

The type of tubes depends on the PCR instrument used for analysis. Use disposable filter tips for adding reagents, DNA and control samples into tubes.

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

1. Calculate the required quantity of each reagent for reaction mixture preparation. For one reaction:

   10 µl of **PCR-mix-FL *HHV7*,**
   5 µl of **PCR-buffer-H**.

Prepare the reaction mixture for the total number of test and control samples plus several extra reactions. See number of control samples in item 7.

NOTE: Prepare the reaction mixture just before use.

2. Thaw the tubes with **PCR-mix-FL *HHV7*** and **PCR-buffer-H.** Thoroughly vortex the tubes with **PCR-mix-FL *HHV7*** and **PCR-buffer-H** and sediment the drops by vortex.
3. In a new tube prepare the reaction mixture. Mix the required quantities of **PCR-mix-FL *HHV7*** and **PCR-buffer-H**. Sediment the drops by vortex.
4. Take the required number of the tubes or strips taking into account the number of test samples and control samples.
5. Transfer **15 µl** of the prepared reaction mixture to each tube. Discard the unused reaction mixture.
6. Add **10 µl** of **DNA samples** extracted from test samples at the DNA extraction stage using tips with filter.

NOTE: Avoid transferring the sorbent together with the RNA samples extracted with the reagent kit for extraction magnetic separation.

7. Carry out the control reactions:

C1	–	Add **10 µl** of **C1 *HHV7*** to the tube labeled **C1**.
C2	–	Add **10 µl** of ***C2 HHV7*** to the tube labeled **C2**.
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 *HHV7* reagent** to the tube labeled PCE (Positive control of Extraction).
NOTE:		It is also necessary to carry out Negative Control of Amplification (NCA) at suspicion on possible contamination
NCA	–	Add **10 µl** of **TE-buffer** to the tube with reaction mixture.

8.2.2 Amplification

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

Table 3

eSens unified amplification program

	Rotor-type Instruments (e.g Rotor-Gene Q or equivalent)		**Plate-type Instruments** (e.g CFX 96 Touch, CFX 96 Opus, QuantStudio 5 or equivalent)	
Step	**Temperature, °C**	**Time**	**Florescent signal detection**	**Cycles**
1	**50**	**15 min**	**–**	**1**
2	**95**	**15 min**	**–**	**1**
3	**95**	**10 s**	**–**	**45**
	60	**20 s**	**FAM, JOE, ROX**	

NOTE: Any combination of the tests (including tests with reverse transcription and amplification) can be performed in one instrument simultaneously with the use of the unified amplification program. If several tests in "multiprime" format are carried out simultaneously, the detection is enabled in other used channels except for the specified ones. If in one instrument only the tests for the pathogen DNA detection are carried out simultaneously, the first step of reverse transcription (50 °C – 15 min) can be omitted for time saving.

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

2. Adjust the fluorescence channel sensitivity according to the *Technical Sheet*.
3. Insert tubes into the reaction module of the device.

NOTE: It is recommended to sediment drops from walls of tubes by short centrifugation (1-3 s) before placing them into the instrument.
Insert empty tubes at the edges of reaction module in case of incomplete filling of plate-type instrument.

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 three channels:

Table 4

Channel for the fluorophore	**FAM**	**JOE**	**ROX**
Amplification product	IC Glob DNA	*HHV7* DNA	Internal Control-FL (IC) DNA

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.

Based on the obtained *Ct* values and specified concentration values of DNA calibrators (C1 and C2) a calibration line is plotted and the concentration values of *HHV7* DNA, human DNA (IC Glob) and Internal Control-FL (IC) DNA in copies/reaction are calculated. *HHV7* DNA quantity per 1 ml is calculated according to the formula:

$$\frac{\textbf{number of } \boldsymbol{HHV7} \textbf{ DNA copies per reaction}}{\textbf{number of Internal Control} - \textbf{FL (IC) DNA copies per reaction}} \times \mathbf{A} \times \mathbf{B} = \textbf{copies / ml}$$

where;

A is the coefficient taking into account the volume of extraction. It is calculated by the formula:

$$\mathbf{A} = \frac{\mathbf{100}}{\textbf{extraction volume } (\mu\textbf{l})}$$

B is the number of copies of IC in 1 ml of the test sample. The coefficient takes into account the DNA loss during the extraction procedure.

When DNA is extracted from whole blood samples, the obtained *HHV7* DNA concentration values can be normalized to the standard number of human cells (the number of *HHV7* copies per 10^5 of human cells). Normalized *HHV7* DNA concentration values are calculated according to the formula:

$$\mathbf{lg}\left(\frac{\textbf{number of } \boldsymbol{HHV7} \textbf{ DNA copies per reaction}}{\textbf{number of human DNA copies per reaction}} \times \mathbf{2} \times \mathbf{10^5}\right) = \mathbf{lg}($$

$$\textbf{number of } \boldsymbol{HHV7} \textbf{ DNA copies per } \mathbf{10^5} \textbf{ of human cells})$$

Normalized concentration values reflect the number of human cells of the pathogen relative to human cells. The value of the concentration of human DNA allows you to assess the quality of taking biological material.

NOTE: The values of calibrators' concentrations and coefficient B are specified in the *Technical Sheet* enclosed to the given lot of PCR kit and couldn't be used for result calculation in analysis with the use of another lot reagents.

NOTE: It is allowed to use the results obtained for DNA calibrators in the previous run on this instrument for subsequent runs with the given lot of **eSens HHV7- screen/monitor-FRT PCR kit**. For that purpose export the results of DNA calibrators using the software of the instrument.

Table 5

Results Interpretation for the test samples

Result	Interpretation
Invalid	The *Ct* value in the channel for the ROX fluorophore is absent or determined greater than the boundary value. The PCR analysis (beginning with the DNA extraction stage) should be repeated for this sample
Invalid (for the whole blood analysis only)	IC Glob DNA concentration is less than 2,000 copies/reaction and the value of calculated concentration is absent in the channel for the JOE fluorophore. The PCR analysis (beginning with the DNA extraction stage) should be repeated for this sample. If IC Glob DNA is absent in the test sample it is necessary to repeat sampling and PCR analysis
Invalid (for the oropharyngeal swab analysis only)	IC Glob DNA concentration is less than 500 copies/reaction and the value of calculated concentration is absent in the channel for the JOE fluorophore. The PCR analysis (beginning with the DNA extraction stage) should be repeated for this sample. If IC Glob DNA is absent in the test sample it is necessary to repeat sampling and PCR analysis
HHV7 DNA is not detected	The *Ct* value for *HHV7* DNA is absent and the *Ct* value determined in the channel for the ROX fluorophore is less than the boundary value
less than 500 *HHV7* DNA copies/ml	The concentration of detected *HHV7* DNA is less than the lower limit of measurement range of the PCR kit
X x 10^y *HHV7* DNA copies/ml	The concentration of detected *HHV7* DNA falls within the measurement range of the PCR kit
greater than $1x10^7$ *HHV7* DNA copies/ml	The concentration of detected *HHV7* DNA is greater than the upper limit of measurement range of the PCR kit. If the accurate quantification is required, the extracted sample is to be diluted by TE-buffer reagent (for example, 100-fold dilution) and the PCR-analysis is to be repeated from the amplification stage. The result obtained after repeated analysis should be multiplied by the coefficient of the sample dilution

The result of the analysis is considered reliable only if the results obtained for the controls of extraction and amplification are correct (see Table 6 and enclosed *Technical Sheet*).

Table 6

Results for controls

Control	Stage for control	*Ct* value in the channel for fluorophore		
		FAM	**JOE**	**ROX**
PCE	DNA extraction	< boundary value	< boundary value; concentration value is within the range	< boundary value
C–	DNA extraction	Absent	Absent	< boundary value
NCA	PCR	Absent	Absent	Absent
C1	PCR	*Ct* value and calculated concentration are determined	*Ct* value and calculated concentration are determined	*Ct* value and calculated concentration are determined
C2	PCR	*Ct* value and calculated concentration are determined	*Ct* value and calculated concentration are determined	*Ct* value and calculated concentration are determined

NOTE: Boundary *Ct* values and the concentration range of Positive Control *HHV7* are specified in the *Technical Sheet* enclosed to the PCR kit.

10 TROUBLESHOOTING

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

1. The *Ct* value determined for the Positive Control of Extraction (PCE) in the channels for the FAM and/or JOE and/or ROX fluorophores is greater than the boundary *Ct* value or absent. The PCR analysis (beginning with the DNA extraction stage) should be repeated for all samples.
2. The calculated concentration of the Positive Control *HHV7* does not fit in the range specified in the bulletin. The PCR analysis (beginning with the DNA extraction stage) should be repeated for all samples.
3. For the Negative Control of Extraction (C–):

   a) The *Ct* value is determined in the channels for the FAM and/or JOE fluorophores. The contamination of laboratory with amplification fragments or contamination of reagents, test samples is probable at any stage of PCR analysis. Measures for detecting and elimination of contamination source must be taken. The PCR analysis (beginning with the DNA extraction stage) should be repeated for all samples in which specific DNA was detected;

   b) The *Ct* value is absent or more than the boundary value in the ROX fluorophore channel. This means that the Negative Control of Extraction (C–) did not perform the contamination control function. The PCR analysis (beginning with the DNA extraction stage) should be repeated for all samples in which DNA of the analyzed microorganisms was detected.

4. The *Ct* value is determined for the Negative Control of amplification (NCA) in the channels for the FAM and/or JOE and/or ROX fluorophores. The contamination of laboratory with amplification fragments or contamination of reagents, test samples is probable at any stage of PCR analysis. Measures for detecting and elimination of contamination source must be taken. The amplification and detection should be repeated for all samples in which specific DNA was detected.
5. The *Ct* values are absent for the DNA-calibrators C1 and C2 in either of the specified channels for fluorophores. The amplification and detection should be repeated for all the samples.
6. The correlation coefficient R^2 is less than 0.98 when plotting the calibration curve. Check the correctness of set concentrations of calibrators in accordance with the bulletin. If the improper result has been obtained again the amplification and detection for all the samples should be repeated.
7. The *Ct* value is determined for the test sample, whereas the area of typical exponential growth of fluorescence is absent (the graphic looks like approximate straight line). It is necessary to check the correctness of selected threshold line level or parameters of base line calculation. If the result has been obtained with the correct level of threshold line (base line), the amplification and detection should be repeated for this sample.

11 TRANSPORTATION

eSens HHV7 QT PCR kit should be transported at 2–8 °C for no longer than 5 days. PCR kit can be transported at 2–25 °C for no longer than 3 days.

12 STABILITY AND STORAGE

All components of the **eSens HHV7 QT PCR kit** are to be stored at 2–8 °C when not in use (except for PCR-buffer-H and PCR-mix-FL *HHV7*). All components of the **eSens HHV7 QT PCR kit** are stable until the expiry date stated on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

NOTE: PCR-buffer-H and PCR-mix-FL *HHV7* are to be stored at the temperature from minus 24 to minus 16 °C

NOTE: PCR-mix-FL *HHV7* is to be kept away from light

13 SPECIFICATIONS

13.1 Analytical sensitivity and linear range

Table 7

Biological material	Transport medium	The volume of sample for extraction, µl	Nucleic acid extraction kit	PCR kit	Limit of detection, copies/ml	Linear measurement range, copies/ml
Blood plasma	—	100	RIBO-prep	ES3207A	200	500 – $1x10^7$
	—	200	ePure Viral Nucleic Acid Extraction Kit			
Whole blood	—	100	RIBO-prep			
Saliva	—	100	RIBO-prep			
Oropharyngeal swab	Transport Medium for Storage and Transportation of Respiratory Swabs	100	RIBO-prep			
Cerebrospinal fluid	—	100	RIBO-prep			
	—	200	ePure Viral Nucleic Acid Extraction Kit			

13.2 Analytical specificity

The analytical specificity of **eSens HHV7 QT PCR kit** is ensured by the selection of specific primers and probes as well as stringent reaction conditions. The primers and probes have been checked for possible homologies to all sequences published in gene banks by sequence comparison analysis.

The PCR kit detects the DNA fragment of *HHV7* (clinical sample with the concentration of *HHV7* no less than 10^4 copies/ml, specificity confirmed by direct sequencing of nucleotide sequences).

The analytical specificity was proved by investigation of the human DNA and DNA/RNA of the following microorganisms/strains:

- Strains of *Human gammaherpesvirus* 4 NIBSC No. 09/260, *Human polyomavirus* 1 NIBSC No. 14/212, *Human polyomavirus* 2 NIBSC No. 14/114, *Primate erythroparvovirus* 1 NIBSC No. 99/802, *Human betaherpesvirus* 5 NIBSC No. 09/162 from the NIBSC collection (National Institute for Biological Standards and Control, UK) at a concentration of at least $5x10^5$ IU/ml;
- Strains of *Streptococcus pyogenes* ATCC 19615™, *Streptococcus agalactiae* ATCC 12386™, *Listeria monocytogenes* ATCC 7644™, *Neisseria meningitidis* ATCC 13102™, *Haemophilus influenzae* ATCC 33930™, *Staphylococcus aureus* ATCC 6538P™ from the ATCC collection (American Type Culture Collection, USA) at a concentration of at least $1x10^7$ copies/ml;
- Clinical isolates of a panel of strains and isolates held by the Federal Budgetary Scientific Institution of the Central Research Institute of Epidemiology of Rospotrebnadzor: *Enterovirus* spp., *Human alphaherpesvirus* 1, *Human alphaherpesvirus* 2, *Human alphaherpesvirus* 3,

Human betaherpesvirus 6A, *Human betaherpesvirus* 6B, *Rubella virus*, *Human respiratory syncytial virus*, *Human metapneumovirus*, *Human parainfluenza virus* types 1-4, *Human coronavirus* (NL-63, 229E, HKU-1, OC43), *Human rhinovirus*, *Human adenovirus* B, C, E, *Human bocavirus*, *Influenza virus* A, *Influenza virus* B at a concentration of at least $1x10^4$ copies/ml;

- Human DNA (Sigma Aldrich, USA) at a concentration of at least $1x10^6$ copies/ml.

The nonspecific responses were not observed while testing the DNA samples of the above mentioned microorganisms, as well as human DNA.
The clinical specificity of **eSens HHV7 QT PCR kit** was confirmed in laboratory clinical trials.
The information about known interfering substances is specified in the *Interfering substances and limitations of using test material samples*.

13.3 Repeatability and reproducibility

Repeatability and reproducibility were determined by testing of negative blood plasma in which *HHV*7 DNA was not previously detected and then a quality control sample (QCS) containing *HHV*7 DNA has been added to final concentrations of $1x10^6$; $1x10^5$ and $1x10^4$ copies/ml.
Repeatability conditions included testing in the same laboratory, by the same operator, using the same equipment within a short period of time. Reproducibility conditions included testing different lots of reagent kit in different laboratories, by different operators, in different days, using different equipment.

Table 8

Repeatability

Nucleic acid extraction kit	Initial concentration value, copies/ml	Number of repeats	Average concentration value, lg	Standard deviation (SD)	Coefficient of variation (CV), %
RIBO-prep	$1x10^6$	10	5.9	0.03	0.4
	$1x10^5$	10	5.0	0.02	0.5
	$1x10^4$	10	4.0	0.04	0.9
ePure Viral Nucleic Acid Extraction Kit	$1x10^6$	10	6.1	0.03	0.5
	$1x10^5$	10	5.3	0.08	1.4
	$1x10^4$	10	4.2	0.09	2.1

Table 9

Reproducibility

Nucleic acid extraction kit	Initial concentration value, copies/ml	Number of repeats	Average concentration value, lg	Standard deviation (SD)	Coefficient of variation (CV), %
RIBO-prep	$1x10^6$	80	6.0	0.08	1.3
	$1x10^5$	80	5.0	0.10	2.1
	$1x10^4$	80	4.0	0.09	2.3
ePure Viral Nucleic Acid Extraction Kit	$1x10^6$	80	6.1	0.14	2.4
	$1x10^5$	80	5.1	0.20	4.0
	$1x10^4$	80	4.1	0.16	4.0

13.4 Trueness

The trueness was determined by testing negative blood plasma samples in which *HHV*7 DNA was not previously detected and then quality control sample (QCS) containing *HHV*7 DNA has been added to a final concentration of $1.3x10^6$ copies/ml.

Table 10

Trueness

Micro-organism	Number of repeats	Average value of measurement, lg	Specified value, lg	Bias (B), %
*HHV*7	25	6.00	6.00	0.00

13.5 Diagnostic characteristics

Samples of biological material (namely: 180 whole blood samples, 180 saliva samples and 180 oropharyngeal swabs) from children with primary infection aged from one to three years with a confirmed B08.2 Exanthema subitum (sixth disease) diagnosis according to International Classification of Diseases, 10th revision (ICD 10) were used to determine the diagnostic characteristics of the PCR kit. 180 negative samples of the cerebrospinal fluid and 180 negative samples of blood plasma with the addition of the *HHV7* DNA quality control sample to final concentrations of *HHV7* DNA from 500 to $1x10^7$ copies/ml were tested to confirm the diagnostic sensitivity. Blood plasma, whole blood, saliva, oropharyngeal swabs (180 samples of each material) taken from conventionally healthy blood donors, as well as 180 samples of cerebrospinal fluid from patients without viral infection were used to confirm the diagnostic specificity.

QX100 droplet digital PCR (ddPCR) system (Bio-Rad Laboratories, Inc., USA) was used as the reference assay.

The results are specified in tables 11 and 12.

Table 11

The results of testing eSens HHV7 QT PCR kit in comparison with the reference assay

Sample type	The results of application of eSens HHV7 QT PCR kit		Results of using the reference assay	
			Positive	Negative
Blood plasma	360 samples were tested	**Positive**	180	0
		Negative	0	180
Whole blood	360 samples were tested	**Positive**	180	0
		Negative	0	180
Saliva	360 samples were tested	**Positive**	180	0
		Negative	0	180
Oropharyngeal swab	360 samples were tested	**Positive**	180	0
		Negative	0	180
Cerebrospinal fluid	360 samples were tested	**Positive**	180	0
		Negative	0	180

Table 12

Diagnostic characteristics of eSens HHV7 QT PCR kit

Sample type	Diagnostic sensitivity* (with a confidence level of 95 %)	Diagnostic specificity** (with a confidence level of 95 %)
Blood plasma	100 (98.3 – 100) %	100 (98.3 – 100) %
Whole blood	100 (98.3 – 100) %	100 (98.3 – 100) %
Saliva	100 (98.3 – 100) %	100 (98.3 – 100) %
Oropharyngeal swab	100 (98.3 – 100) %	100 (98.3 – 100) %
Cerebrospinal fluid	100 (98.3 – 100) %	100 (98.3 – 100) %

***** Relative sensitivity in comparison with applied reference assay.

****** Relative specificity in comparison with applied reference assay.

14 QUALITY CONTROL

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

15 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	**C1**, **C2**	DNA-calibrators
PCE	Positive control of extraction	**IC**	Internal control

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
01_04/2022		

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