ECOLI

eSens

CE

IVD

For Professional Use Only

eSens EBV/HHV6 QT PCR kit

REF ES3240A

Instructions for Use

INTENDED USE

1 **eSens EBV/HHV6 QT PCR kit** is an *in vitro* nucleic acid amplification test for qualitative and quantitative detection of *Epstein-Barr virus* (*EBV*) DNA and *Human Herpes virus* type 6 (*HHV6*) DNA in clinical material (whole venous blood, white blood cells, cerebrospinal fluid, saliva, amniotic liquid, bronchoalveolar lavage fluid, plasma of venous and tissue (biopsy, surgical, autopsy) using real-time hybridization-fluorescence detection of amplified products.

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

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PRINCIPLE OF PCR DETECTION

Principle of testing is based on the DNA extraction from the samples of test material and the simultaneous amplification of DNA fragments of the detected microorganism and DNA of the human β-globin gene with hybridization-fluorescence detection. DNA of the β-globin gene is used as an endogenous internal control (IC Glob) and allows not only to control all stages of the PCR study for each sample, but also to evaluate the adequacy of the material and its storage.

Amplification of DNA fragments with the use of specific primers and Taq-polymerase enzyme are performed with the DNA 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.

eSens EBV/HHV6 QT PCR kit uses “hot-start”, which greatly reduces the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by using 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 deoxyuridine triphosphate (dUTP).

The results of amplification are registered in the following fluorescence channels.

Table 1

Channel for fluorophore	FAM	JOE	Cy5
DNA-target	IC Glob DNA	*EBV* DNA	*HHV*6 DNA
Target gene	β-globin gene	LMP-gene	DNA polymerase catalytic subunit

CONTENT

eSens EBV/HHV6 QT PCR kit (ES3240A) includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-1-FRT *EBV* / *HHV*6 / Glob	clear liquid from colorless to light lilac colour	0.6	2 tubes
PCR-buffer-H	colorless clear liquid	0.3	2 tubes
TE-buffer	colorless clear liquid	0.2	1 tube
DNA calibrator KSG1	colorless clear liquid	0.2	1 tube
DNA calibrator KSG2	colorless clear liquid	0.2	1 tube
Negative Control (C-)*	colorless clear liquid	1.2	4 tubes
Positive Control DNA *EBV* / *HHV*6 and human DNA**	colorless clear liquid	0.5	1 tube

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

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

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

ADDITIONAL REQUIREMENTS

For pretreatment

- Reagent for pretreatment of whole venous or cord blood
- Disposable screwed or tightly closed 1.5-ml tubes

For DNA extraction and amplification

- DNA extraction kit.
- 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 Q (QIAGEN, Germany), CFX 96 Touch, CFX 96 Opus (Bio-Rad, USA), QuantStudio 5 (Thermo Fisher Scientific), or equivalent).
- Disposable polypropylene tubes:
  a) thin-walled 0.2-ml PCR tubes with optical transparent domed or flat caps if a plate-type instrument is used;

b) 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.

- Pipettes (adjustable).
- 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.

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 a biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all samples or reagent spills using a disinfectant, such as 0.5 % sodium hypochlorite or other suitable disinfectant.
- Avoid inhalation of vapors, samples and reagents contact with the skin, eyes, and mucous membranes. Harmful if swallowed. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice if necessary.
- While observing the conditions of transportation, operation and storage, there are no risks of explosion and ignition.
- 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.

SAMPLING AND HANDLING

eSens EBV/HHV6 QT PCR kit is intended for analysis of the DNA extracted with DNA extraction kits from the clinical material (whole venous blood, white blood cells, saliva, amniotic liquid, bronchoalveolar lavage fluid, cerebrospinal fluid, plasma of venous and tissue (biopsy, surgical, autopsy).

6 WORKING CONDITIONS

eSens EBV/HHV6 QT PCR kit should be used at the temperature from 20 to 28 °C and relative humidity from 15 to 75 %.

7 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)

In the extraction procedure it is necessary to carry out the control reactions as follows:

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 *EBV* / *HHV6* and human DNA** to the tube labelled PCE (Positive Control of Extraction).

NOTE: Extract the DNA according to the manufacturer's protocol.

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 to prepare the reaction mixture. For one reaction 10 µl of PCR-mix-1-FRT *EBV* / *HHV*6 / Glob and 5 µl of PCR-buffer-H is required. Prepare the mixture for total number of test and control samples (see numbers of control samples in item 4) plus one extra reaction (see Table 2).

Table 2

Scheme of reaction mixture preparation

		Volume of reagents for the specified number of reactions, µl	
Reagent volume for 1 reaction (µl)		**10.0**	**5.0**
Quantity of test samples		**PCR-mix-1-FRT *EBV / HHV6* / Glob**	**PCR-buffer-H**
For quantitative analysis	**For qualitative analysis**		
1	**4**	**90**	**45**
2	**5**	**100**	**50**
3	**6**	**110**	**55**
4	**7**	**120**	**60**
5	**8**	**130**	**65**
6	**9**	**140**	**70**
7	**10**	**150**	**75**
8	**11**	**160**	**80**
9	**12**	**170**	**85**
10	**13**	**180**	**90**
11	**14**	**190**	**95**
12	**15**	**200**	**100**
13	**16**	**210**	**105**
14	**17**	**220**	**110**
15	**18**	**230**	**115**
16	**19**	**240**	**120**
17	**20**	**250**	**125**
18	**21**	**260**	**130**
19	**22**	**270**	**135**
20	**23**	**280**	**140**
21	**24**	**290**	**145**
22	**25**	**300**	**150**
23	**26**	**310**	**155**
24	**27**	**320**	**160**
25	**28**	**350**	**165**
30	**33**	**380**	**190**

NOTE: Prepare the reaction mixture just before use.

2. Thaw the test tube with **PCR-mix-1-FRT EBV / HHV6 / Glob**. Mix the contents of the tubes with **PCR-mix-1-FRT EBV / HHV6 / Glob** and **PCR-buffer-H,** sediment the drops by vortex.
3. Prepare the reaction mixture in a separate test tube. Mix the required amount of **PCR-mix-1-FRT EBV / HHV6 / Glob** and **PCR-buffer-H,** and sediment the drops by vortex.
4. Take the required number of tubes or strips for amplification of test and control DNA samples.
5. Transfer **15 µl** of the prepared mixture into each tube. Discard the unused reaction mixture.
6. Add **10 µl** of **DNA** obtained at the DNA extraction stage to the tubes with the reaction mixture.
7. Carry out the control reactions:

<u>For qualitative analysis:</u>

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

C+ – Add **10 µl** of **DNA calibrator КSG2** to the tube labeled C+ (Positive Control of Amplification).

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

PCE – Add **10 µl** of **the sample extracted from the Positive Control DNA *EBV* / *HHV6* and human DNA reagent** to the tube labeled PCE (Positive control of Extraction).

<u>For quantitative analysis:</u>

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

Calibrator K1 – Add **10 µl** of **DNA calibrator КSG1** to two tubes labeled **K1**.
Calibrator K2 – Add **10 µl** of **DNA calibrator КSG2** to two tubes labeled **K2**.

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

PCE – Add **10 µl** of **the sample extracted from the Positive Control DNA *EBV* / *HHV6* 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 unified amplification program

Step	Temperature, °C	Time	Fluorescence detection	Cycles
1	**50**	**15 min**	**-**	**1**
2	**95**	**15 min**	**-**	**1**
3	**95**	**10 s**	**-**	**45**
	60	**20 s**	**FAM, JOE, Cy5**	

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 only the tests for DNA detection are performed in one instrument then the first step of reverse transcription (50 °C – 15 min) can be omitted for time saving.

Table 4

eSens-1 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**	**Cycles**	**Temperature, °C**	**Time**	**Cycles**
1	**95**	**15 min**	**1**	**95**	**15 min**	**1**
2	**95**	**5 s**	**5**	**95**	**5 s**	**5**
	60	**20 s**		**60**	**20 s**	
	72	**15 s**		**72**	**15 s**	
3	**95**	**5 s**	**40**	**95**	**5 s**	**40**
	60	**20 s fluorescence detection**		**60**	**30 s fluorescence detection**	
	72	**15 s**		**72**	**15 s**	

Fluorescent signal is detected in the channels for the **FAM, JOE** and **Cy5** fluorophores (if other tests are performed simultaneously, the detection is assigned in other used channels).

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.

DATA ANALYSIS

Fluorescence signal accumulation curves indicating the accumulation of the amplification product are analyzed in three channels:

Table 5

Channel for the fluorophore	**FAM**	**JOE**	**Cy5**
Amplification product	β-globin gene DNA fragment (IC Glob)	*EBV* DNA	*HHV*6 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.

Qualitative analysis

Principle of the interpretation is the following.

Table 6

Results interpretation

***Ct* value in the channel for the fluorophore**			**Result**
FAM	**JOE**	**Cy5**	
< boundary value*	< boundary value	determined or absent	*EBV* DNA **is detected**
< boundary value*	determined or absent	< boundary value	*HHV*6 DNA **is detected**
< boundary value*	absent	absent	*EBV* DNA and *HHV6* DNA **are not detected**
absent or > boundary value	determined or absent	determined or absent	**Invalid* result**
< boundary value*	> boundary value	> boundary value	**Equivocal****

* For samples of saliva, cerebrospinal fluid, amniotic fluid, bronchoalveolar lavage fluid and plasma of venous, the absence or exceeding of the boundary *Ct* value **is ALLOWED**.

** In case of **invalid** result, it is necessary to repeat PCR-analysis of the corresponding test sample starting from the DNA extraction stage (if the *Ct* value in the channel for FAM fluorophore is greater than the boundary value) or to repeat the biological material sampling and PCR-analysis (if the *Ct* value of the channel for FAM fluorophore is absent).

*** In case of **equivocal** result, it is necessary to repeat PCR-analysis of the corresponding sample in two repeats, starting from the DNA extraction stage. If a reproducible positive *Ct* value is obtained, the result is considered **positive**.

NOTE: Boundary *Ct* values are specified in the *Technical Sheet* enclosed to the PCR kit.

The result of the PCR analysis is considered reliable only if the results obtained for controls of extraction and amplification stages are correct (according to the Table 7 and the *Technical Sheet* enclosed to the PCR kit*).*

Table 7

Results for controls in qualitative analysis

Control	Stage for control	*Ct* in the channel for fluorophore		
		FAM	JOE	Cy5
PCE	DNA extraction	<boundary value	<boundary value	<boundary value
C–	DNA extraction	Absent	Absent	Absent
NCA	PCR	Absent	Absent	Absent
C+	PCR	<boundary value	<boundary value	<boundary value

For quantitative analysis, based on the set values of DNA-calibrator concentration and the obtained *Ct* values, the calibration line is automatically drawn and the concentration of *EBV* DNA, *HHV6* and human DNA (IC Glob) in copies/reaction are calculated. The obtained values are used to calculate the number of copies of *EBV* DNA and *HHV6* DNA in 1 ml of test samples of all types of biological material:

number of viral DNA copies per reaction x 100 x A = copies/ml

where:

A – coefficient, taking into account the extraction volume, is calculated by the formula:

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

For samples of whole venous blood, leukocytes of venous, tissue (biopsy, surgical, autopsy) material the obtained values of *EBV* DNA and *HHV6* DNA concentration in copies per reaction can be normalised to the standard number of human cells (number of copies of *EBV* DNA and *HHV6* DNA per 10^5 human cells). Calculation of normalised concentration values of *EBV* DNA and *HHV6* DNA is performed according to the following formulas:

$$\mathbf{lg}\left\{\frac{\mathbf{number\ of\ viral\ DNA\ copies\ in\ PCR\ sample}}{\mathbf{number\ of\ Glob\ DNA\ copies\ in\ PCR\ sample}}\ \mathbf{x\ 2\ x\ 10^5}\right\} = \mathbf{lg}\{\mathbf{viral\ DNA\ copies/10^5\ cells}\}$$

NOTE: The normalised concentration values reflect the number of pathogen cells relative to the number of human cells. In addition, the human DNA concentration value allows the quality of biological material collection to be assessed.

A conversation factor is used to calculate the normalised values: $2x10^5$ human genomes = 10^5 cells.

NOTE: Concentration values of DNA-calibrators are specified in the *Technical Sheet* enclosed to the PCR kit.

Principle of interpretation is the following:

Table 8

Results Interpretation for the test samples (quantitative analysis)

Result	Interpretation
Invalid – concentration cannot be calculated (when studying whole venous blood, leukocytes from venous, tissue (biopsy, surgical, autopsy) material)	IC Glob DNA concentration is less than 2000 copies/ml. It is necessary to repeat the PCR-analysis of this sample starting from DNA extraction stage. If IC Glob DNA is absent in the test sample, biological material sampling and PCR-analysis are to be repeated.
Invalid – concentration calculation is not possible (for oropharyngeal swabs)	IC Glob DNA concentration is less than 500 copies/ml. It is necessary to repeat the PCR-analysis of this sample starting from DNA extraction stage. If IC Glob DNA is absent in the test sample biological material sampling and PCR-analysis are to be repeated
EBV DNA and *HHV6* DNA are not detected	The *Ct* value for *EBV* DNA and *HHV6* DNA is absent and the IC Glob concentration in the channel for FAM fluorophore is greater than boundary value*
Less than $1x10^3$ (when extracted from 100 and 200 μl of sample) and less than 300 (when extracted from 1000 μl of sample) copies *EBV* DNA/ml and/or *HHV6* DNA/ml	*EBV* DNA and/or *HHV6* DNA is detected at a concentration less than the lower limit of the measurement range of the PCR kit.
X x 10^y copies *EBV* DNA/ml and/or *HHV6* DNA/ml	*EBV* DNA and/or *HHV6* DNA is detected at a concentration within the measurement range of the PCR kit.
More than $1x10^7$ copies *EBV* DNA/ml and/or *HHV6* DNA/ml	*EBV* DNA and/or *HHV6* DNA is detected at a concentration greater than the upper limit of the measurement range of the PCR kit. If an accurate quantitative result is required, dilute the DNA sample with TE-buffer reagent (e.g. 10 times) and repeat the PCR-analysis from the amplification stage. The result obtained in the repeat test should be multiplied by the sample dilution factor

NOTE: For saliva, venous plasma, cerebrospinal fluid, amniotic fluid transudate and bronchoalveolar lavage the quantity of IC Glob DNA less than 500 copies/reaction is **acceptable**.

The result of the PCR analysis is considered reliable only if the results obtained for controls of extraction and amplification stages are correct (according to the Table 9 and the *Technical Sheet* enclosed to the PCR kit*).*

Table 9

Results for controls in quantitative analysis

Control	Stage for control	*Ct* in the channel for fluorophore		
		FAM	JOE	Cy5
PCE	DNA extraction	concentration value falls in the range	concentration value falls in the range	concentration value falls in the range
C–	DNA extraction	*Ct* value is absent	*Ct* value is absent	*Ct* value is absent
NCA	PCR	*Ct* value is absent	*Ct* value is absent	*Ct* value is absent
KSG1, KSG2	PCR	*Ct* value and calculated concentration are defined	*Ct* value and calculated concentration are defined	*Ct* value and calculated concentration are defined

Note: The *Ct* boundary values and concentration range of **Positive Control DNA *EBV* / *HHV6* and human DNA** are specified in the *Technical Sheet* enclosed to the PCR kit.

TROUBLESHOOTING

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

1. If any *Ct* value appears in the channels for the FAM, JOE and Cy5 fluorophores for the Negative Control of Amplification (NCA) and Negative Control of Extraction (C–) these results testify the presence of contamination of reagents or samples. In that case the PCR-analysis should be repeated (beginning with the extraction stage) for all samples, in which DNA was detected.
2. If the *Ct* value is absent or greater than the boundary value in the results grid for the Positive Control of Amplification (C+) – **KSG2** – for the qualitative analysis in the channels for the JOE, FAM or Cy5 fluorophores, the amplification must be repeated for all samples where pathogen agent DNA was not detected.
3. If the *Ct* value is absent or greater than the boundary value for the Positive Control of Extraction (PCE) – **Positive Control DNA *EBV* / *HHV6* and human DNA –** in the channels for the JOE, FAM, or Cy5 fluorophores, the results of analysis must be considered as **invalid** for all samples. PCR should be repeated for all samples.
4. If the *Ct* value for given sample was not defined or the *Ct* value exceeds the boundary value in the channel for the JOE, or Cy5 fluorophores, and *Ct* value defined in the channel for the FAM fluorophore exceeds the maximal value specified for IC, the experiment needs to be repeated, starting with the extraction stage. Possible reason is an error in the clinical material pretreatment procedure that leads to the DNA loss or the presence of PCR inhibitors.
5. If the *Ct* value for the clinical samples exceeds the maximal boundary value in the channel for the JOE or Cy5 fluorophore, the results of analysis must be considered as **equivocal**. In that case, it is necessary to conduct additional analysis for that DNA sample with two repeats. If the repeated positive *Ct* value is obtained, the result is considered positive. If the positive *Ct* value can't be reproduced in two repeats, the result is considered **equivocal**.
6. If in quantitative analysis the copies/reaction values in calibrators differ by more than 30 % from the set values, it is necessary to check the tube order in the rotor (calibrators should be placed

in the wells indicated as ***Standard*** in sample table, concentration should correspond to concentration specified in the *Technical Sheet*, well no.1 must be filled with some test tube (not empty)).

7. If the correlation coefficient R in ***Standard Curve*** window is less than 0.9 (in case of quantitative analysis), it means that calibration failed. Check the settings of calibrators and correct inaccuracies, if no effect, repeat PCR for all samples and calibrators.

TRANSPORTATION

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

STABILITY AND STORAGE

10 All components of the **eSens EBV/HHV6 QT PCR kit** are to be stored at 2–8 °C when not in use (except for PCR-mix-1-FRT *EBV* / *HHV*6 / Glob and PCR-buffer-H). All components of the **eSens EBV/HHV6 QT**
11 **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 *EBV* / *HHV6* / Glob and PCR-buffer-H are to be stored at the temperature from minus 24 to minus 16 °C.

NOTE: PCR-mix-1-FRT *EBV* / *HHV6* / Glob is to be kept away from light.

SPECIFICATIONS

12

12.1 Analytical sensitivity and linear range

Clinical material	**Nucleic acid extraction kit**	**Analytical sensitivity**	**Linear measurement range, copies/ml**
Whole venous blood, white blood cells, tissue (biopsy, surgical, autopsy material), cerebrospinal fluid (liquor), plasma of venous, amniotic liquid	RIBO-prep ePure Viral Nucleic acid extraction kit	400 copies/ml	$1x10^3$ – $1x10^7$

12.2 Analytical specificity

eSens EBV/HHV6 QT PCR kit is intended for *Epstein-Barr virus* (*EBV*) DNA and *Human Herpes Virus* type 6 (*HHV*6) DNA. Specific activity of **eSens EBV/HHV6 QT PCR kit** was confirmed by analysis of QCMD panel for *Epstein-Barr virus,* as well as by analysis of clinical material with subsequent confirmation of the results by sequencing the amplified fragments.

13 The activity of the PCR kit components with respect to DNA of other viruses (herpes simplex virus types 1 and 2, 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 was absent.

The clinical specificity of **eSens EBV/HHV6 QT PCR kit** was confirmed in laboratory clinical trials.

QUALITY CONTROL

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

KEY TO SYMBOLS USED

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

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
01_04/2022		
02_08/2024	Through the text	Name of kit was changed: „eSens EBV/HHV6 QT PCR kit PCR kit“ to „eSens EBV/HHV6 QT PCR“.
02_12/2024	Intended use	The list of biological material was expanded.
	Content	Composition was changed.
	Sampling and handling	The information about sampling and handling was expanded.
	Protocol	Working procedure was rewritten.
	Data analysis	Information on the correspondence of the amplification product and channels for the fluorophore , the principle of results interpretationfor the test samples and controls are presented in the tables
	Specifications	The section was rewritten.

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