ECOLI

eSens

CE

IVD

For Professional Use Only

eSens HSV 1/2 genotype QL PCR kit

REF ES3201A

Instructions for Use

1 INTENDED USE

eSens HSV 1/2 genotype QL PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection and typing of *herpes simplex virus* types I and II (HSV I and HSV II) DNA in clinical materials (urogenital, rectal, and pharyngeal swabs; exudate of blisters and erosive-ulcerative lesions of skin and mucous membranes; whole blood; and liquor), taken from the persons suspected of herpes virus infection without distinction of form and presence of manifestation, 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

Herpes simplex virus types I, II DNA detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific *HSV* I and *HSV* II 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 HSV 1/2 genotype QL PCR kit is a qualitative test that contains the Internal Control (Internal Control-FL (IC)). It must be used in the extraction procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition.

eSens HSV 1/2 genotype QL PCR kit uses “hot-start,” which greatly reduces the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by the separation of nucleotides and Taq-polymerase 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. 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 deoxyuridine triphosphate 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**	**ROX**
DNA-target	*HSV* II DNA	*HSV* I DNA	Internal Control (IC)
Target gene	*gpB* gene	*gpB* gene	Artificially synthesized sequence

3 CONTENT

eSens HSV 1/2 genotype QL PCR kit (ES3201A) includes:

Reagent	**Description**	**Volume, ml**	**Quantity**
PCR-mix-1-FL *HSV*-typing	clear liquid from colorless to light lilac colour	1.2	1 tube
PCR-mix-2-FRT	colorless clear liquid	0.3	2 tubes
Polymerase (TaqF)	colorless clear liquid	0.03	2 tubes
Positive Control complex (C+)	colorless clear liquid	0.2	1 tube
DNA-buffer	colorless clear liquid	0.5	1 tube
Negative Control (C–)*	colorless clear liquid	1.2	1 tube
Internal Control-FL (IC)**	colorless clear liquid	1.0	1 tube

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

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

eSens HSV 1/2 genotype QL PCR kit is intended for 110 reactions (including controls).

4 ADDITIONAL REQUIREMENTS

- DNA extraction kit.
- Transport medium.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol barriers (up to 200 µl).
- Tube racks.

- Vortex mixer.
- Desktop centrifuge with a rotor for 2-ml reaction tubes.
- PCR box.
- Personal thermocyclers (for example, Rotor-Gene Q (QIAGEN, Germany); CFX96 (Bio-Rad Laboratories, Inc, USA) or equivalent).
- Disposable polypropylene PCR tubes (0.1- or 0.2-ml):
- 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;
- 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.
- Refrigerator with the temperature range from 2 to 8 °C.
- Deep-freezer with the temperature range from minus 24 to minus 16 °C.
- Reservoir for used tips.

5 GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Temperature in the laboratory room is from 20 to 28 °C, relative humidity is from 15 to 75 %.
- 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 breathing vapours, 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 if necessary.
- Important note with safety information is 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 HSV 1/2 genotype QL PCR kit is intended for analysis of the DNA extracted with use of DNA extraction kits from the clinical material (urogenital, rectal, and pharyngeal swabs; exudate of blisters and erosive-ulcerative lesions of skin and mucous membranes; whole blood; and liquor).

7 WORKING CONDITIONS

eSens HSV 1/2 genotype QL 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

  - **DNA-sorb-AM** (K1-12-100-CE)

- For the automatic extraction

  - **ePure STD DNA Extraction Kit** (E2007) for urogenital samples
  - **ePure Viral Nucleic acid Extraction Kit** (E2003)

The DNA extraction of each test sample is carried out in the presence of **Internal Control-FL (IC).**

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

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 DNA sample is **10 µl**.

1. Thaw the **PCR-mix-2-FRT** tube. Vortex the tubes with **PCR-mix-1-FL *HSV*-typing, PCR-mix-2-FRT, and polymerase (TaqF)** then sediment the drops by short centrifugation (1-2 s).
2. For N reactions (including 2 controls) add to a new tube:

   10*(N+1) µl of **PCR-mix-1-FL *HSV*-typing,**

   5.0*(N+1) µl of **PCR-mix-2-FRT,**

   0.5*(N+1) µl of **polymerase (TaqF).**

3. Mix the prepared mixture and sediment the drops by short centrifugation (1-2 s).

4. Transfer **15 µl** of the prepared mixture to each tube.
5. Add **10 µl** of **DNA** obtained from clinical or control samples at the DNA extraction stage to the prepared tubes using tips with aerosol filter.
6. Carry out the control amplification reactions:

NCA	Add **10 µl** of **DNA-buffer** to the tube labeled NCA (Negative Control of Amplification).
C+	Add **10 µl** of **Positive Control complex (C+)** 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).

8.2.2 Amplification

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

Table 2

eSens-1M 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	20 s	5	95	20 s	5
	60	20 s		60	20 s	
	72	15 s		72	15 s	
3	95	20 s	40	95	20 s	40
	60	20 s fluorescent signal detection		60	30 s fluorescent signal detection	
	72	15 s		72	15 s	

Fluorescent signal is detected in the channels for the FAM, JOE, and ROX fluorophores (other channels are enabled if several tests are simultaneously carried out in a single run).

2. Adjust the fluorescence channel sensitivity.
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.

8.3 Instrument Settings

Test settings for rotor-type instruments

Channel	Calibrate/Gain Optimisation	Threshold	Dynamic tube	Slope Correct	More Settings/ Outlier Removal
FAM/Green	from 5 FI to 10 FI	0.1	On	Off	0%
JOE/Yellow	from 4 FI to 8 FI	0.1	On	Off	5%
ROX/Orange	from 4 FI to 8 FI	0.1	On	Off	5%

Test settings for plate-type instruments

Note - CFX96 - Set ***Ramp Rate* 2,5 °C/s** by clicking the *Step Options* button for each step of cycling.

Channel	Threshold
FAM JOE/HEX ROX	For each channel in *Log Scale* set the threshold line at the level of 10-20 % of maximum fluorescence obtained for the Positive Control of Amplification (C+) in the last amplification cycle

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:

- The signal of the *HSV* type II DNA amplification product is detected in the channel for the FAM fluorophore.
- The signal of the *HSV* type I DNA amplification product is detected in the channel for the JOE fluorophore.
- The signal of the IC amplification product is detected in the channel for the ROX 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:

- ***HSV* II** DNA is **detected** if the *Ct* value is determined in the results grid in the channel for the FAM fluorophore. Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.
- ***HSV* I** DNA is **detected** if the *Ct* value is determined in the results grid in the channel for the JOE fluorophore. Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.
- ***HSV* I** and ***HSV* II** DNA is **not detected** in a sample if *Ct* value is not determined in the results grid (the fluorescence curve does not cross the threshold line) in the channels for the FAM and JOE fluorophores (fluorescence curve does not cross the threshold line) and if the *Ct* value determined in the results grid in the channel for the ROX fluorophore does not exceed the specified boundary *Ct* value.
- The result is considered to be **invalid** if the *Ct* value is not determined (absent) in the channel for the ROX fluorophore and in the channels for the FAM and JOE fluorophores. In such cases, the PCR analysis should be repeated.

The result of the analysis is considered reliable only if the results obtained for the Positive and Negative Controls of amplification as well as for the Negative Control of extraction are correct (see Table 3 and 4).

Table 3

Results for controls

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

Table 4

Boundary *Ct* values

Sample	**Rotor-type instrument**			**Plate-type instrument**		
	Channel for fluorophore					
	FAM	**JOE**	**ROX**	**FAM**	**JOE**	**ROX**
C+	33	30	33	36	33	36
C-	*Ct* is absent		33	*Ct* is absent		36
NCA	*Ct* is absent			*Ct* is absent		
Test samples	-	-	33	-	-	36

10 TROUBLESHOOTING

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

1. If the *Ct* value determined for the Positive Control of Amplification (C+) in the channels for the **FAM and/or JOE** fluorophores is greater than the boundary *Ct* value or absent, the amplification and detection should be repeated for all samples in which Ct value is absent in the channels for the FAM and/or JOE fluorophores respectively.
2. If the *Ct* value is determined for the Negative Control of Amplification (NCA) and/or Negative Control of Extraction (C–) in the channels for the **FAM or JOE** fluorophores, the PCR analysis should be repeated for all samples in which *Ct* value was determined in the channels for the FAM and/or JOE fluorophores respectively.

11 TRANSPORTATION

eSens HSV 1/2 genotype QL PCR kit should be transported at 2–8 °C for no longer than 5 days.

12 STABILITY AND STORAGE

All components of the **eSens HSV 1/2 genotype QL PCR kit** (except for polymerase (TaqF) and PCR-mix-2-FRT) are to be stored at 2–8 °C when not in use. All components of the **eSens HSV 1/2 genotype QL PCR kit** are stable until the expiration date on the label. PCR kit can be stored without unpacking at 2 to 8 °C for 3 months from the date of manufacture before opening. Once opened, PCR kit should be unpacked in accordance with the storage temperatures for each component. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

NOTE: Polymerase (TaqF) and PCR-mix-2-FRT are to be stored at the temperature from minus 24 to minus 16 °C.

NOTE: PCR-mix-1-FL HSV-typing is to be kept away from light.

13 SPECIFICATIONS

13.1 Sensitivity

Clinical material	**Nucleic acid extraction kit**	**Microorganism**	**Sensitivity, GE/ml***
Urogenital swabs**	DNA-sorb-AM ePure STD DNA Extraction Kit	*HSV* type I	10^3
		HSV type II	10^3

* Genome equivalents of microorganism per 1 ml of the sample placed into transport medium.
** Urogenital swabs placed into **Transport medium with mucolytic agent**.

13.2 Specificity

The analytical specificity of **eSens HSV 1/2 genotype QL 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.

The specificity was proved on the panel of DNA samples of the following microorganisms: *CMV; EBV; HHV* types 6 and 7*; HPV; Gardnerella vaginalis; Lactobacillus* spp.*; Escherichia coli; Staphylococcus aureus; Streptococcus pyogenes; Streptococcus agalactiae; Candida albicans; Mycoplasma hominis; Ureaplasma urealyticum; Ureaplasma parvum; Mycoplasma genitalium; Neisseria flava; Neisseria subflava; Neisseria sicca; Neisseria mucosa; Neisseria gonorrhoeae; Chlamydia trachomatis; Treponema pallidum; Trichomonas vaginalis; Toxoplasma gondii.* Nonspecific responses were absent while testing this panel as well as human DNA samples.

The clinical specificity of **eSens HSV 1/2 genotype QL PCR kit** was confirmed in laboratory clinical trials.

13.3 Diagnostic sensitivity

The diagnostic sensitivity of the **eSens HSV 1/2 genotype QL PCR kit** is 100 %.

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The diagnostic specificity of the **eSens HSV 1/2 genotype QL PCR kit** is 100 %.

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
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
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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