



For Professional Use Only

# eSens HGV QL PCR kit

**REF ES3150B**

## Instructions for Use

### 1 INTENDED USE

**eSens HGV QL PCR kit** is an *in vitro* nucleic acid amplification test for qualitative detection of *hepatitis G virus (HGV)* RNA in clinical material (blood plasma) using real-time hybridization-fluorescence detection.

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

### 2 PRINCIPLE OF PCR DETECTION

*HGV* detection by the polymerase chain reaction (PCR) is based on the *HGV* RNA extraction from blood plasma together with the internal control sample (IC); the reverse transcription of *HGV* RNA and the amplification of the pathogen genome specific region using special *HGV* primers. In real-time PCR, the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes that 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 HGV QL PCR kit** is a qualitative test that contains the Internal Control (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 HGV QL 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 IC amplification product is detected in the channel for the FAM fluorophore. The *HGV* cDNA amplification product is detected in the channel for the JOE fluorophore. The Positive Control of Extraction, **Positive Control HGV-FL-rec**, is detected in the channels for the FAM (IC) and JOE (*HGV*) fluorophores. The Positive Control of Amplification, **Positive Control cDNA HGV-FL (C+<sub>HGV-FL</sub>)**, is a complex control for *HGV* and IC. It is detected in the channels for the FAM (IC) and JOE (*HGV*) fluorophores.

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

**Table 1**

Channel for fluorophore	FAM	JOE
cDNA-target	Internal Control-FL (IC) cDNA	HGV cDNA
Target gene	Artificially synthesized sequence	5'UTR

### 3 CONTENT

eSens HGV QL PCR kit (ES3150B) includes:

Reagent	Description	Volume, ml	Quantity
<b>RT-G-mix-2</b>	colorless clear liquid	0.015	1 tube
<b>RT-PCR-mix-1-FL HGV</b>	clear liquid from colorless to light lilac colour	0.6	1 tube
<b>RT-PCR-mix-2-FEP/FRT</b>	colorless clear liquid	0.3	1 tube
<b>Polymerase (TaqF)</b>	colorless clear liquid	0.03	1 tube
<b>TM-Revertase (MMLv)</b>	colorless clear liquid	0.015	1 tube
<b>Positive Control cDNA HGV-FL (C<sup>+</sup><sub>HGV-FL</sub>)</b>	colorless clear liquid	0.1	1 tube
<b>Buffer for elution</b>	colorless clear liquid	1.2	1 tube
<b>Negative Control (C<sup>-</sup>)*</b>	colorless clear liquid	1.2	1 tube
<b>Positive Control HGV-FL-rec**</b>	colorless clear liquid	0.1	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.

\*\*\* add **10 µl** of **Internal Control ICZ-rec (IC)** during the RNA extraction procedure directly to the sample/lysis solution before the extraction.

eSens HGV QL PCR kit is intended for 55 reactions (including controls).

### 4 ADDITIONAL REQUIREMENTS

- RNA/DNA extraction kit or RNA/DNA extraction automatic station.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile RNase/DNase-free pipette tips with aerosol barriers (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with 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:
  - a) 0.2-ml PCR tubes with optical transparent domed or flat caps if a plate-type instrument is used;
  - b) 0.2-ml PCR tubes with flat caps 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 for used tips.

## 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 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 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.
- 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 HGV QL PCR kit** is intended for the reverse transcription of RNA and amplification of cDNA extracted by RNA/DNA extraction kits from peripheral blood plasma (serum).

### 6.1 Peripheral blood plasma (serum).

Blood samples are taken into the tube with 3% EDTA solution (20 parts of blood to 1 part of EDTA). Closed tubes with blood are turned several times upside down and back again. Blood plasma should be taken and transferred to new tubes within 6 h after taking blood. For this purpose, tubes with blood are centrifuged at 800–1600 g for 20 min. After that blood plasma should be taken and transferred to new disposable tubes.

To obtain serum, tubes with blood should be incubated at room temperature to allow complete clot formation. Then tubes are centrifuged at 800–1600 g for 10 min. After that serum should be taken and transferred to new disposable tubes.

Blood plasma (serum) can be stored unfrozen (at 2–8 °C) for at most 3 days or frozen (at the temperature not more than minus 68° C) for a long time.

## 7 WORKING CONDITIONS

**eSens HGV QL PCR kit** should be used at 18–25 °C.

## 8 PROTOCOL

### 8.1 RNA 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 the automatic extraction

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

**NOTE:** Extract the RNA according to the manufacturer's protocol.

The RNA extraction for each sample is carried out in the presence of **Internal Control ICZ-rec.**

The purified RNA can be stored at 2–8 °C for 4 hours, at the temperature not more than minus 16 °C for one month or at the temperature not more than minus 68 °C for one year.

### 8.2 Preparing the reverse transcription 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.

Total reaction volume is **25 µl**, the volume of RNA sample is **10 µl**.

#### 8.2.1 Preparing tubes for PCR

**NOTE:** All components of the reaction mix should be mixed immediately before use. Mix reagents for the required number of reactions for experimental and control samples according to Table 2.

1. Thaw Before starting work, thaw and thoroughly vortex all reagents of the kit. Make sure that there are no drops on the caps of the tubes.
2. Take the required number of tubes for amplification for the clinical and control samples (two controls of extraction and two controls of amplification). The type of tubes depends on the PCR instrument used for analysis.
3. To prepare the reaction mixture, mix reagents per one reaction in a new sterile tube: **10 µl of RT-PCR-mix-1-FL HGV, 5 µl of RT-PCR-mix-2-FEP/FRT, 0.25 µl of RT-G-mix-2, 0.5 µl of polymerase (TaqF) and 0.25 µl of TM-Revertase (MMIv)**. Thoroughly vortex the mixture, make sure that there are no drops on the caps of the tubes.

Table 2

## Reaction mixture preparation

Reagent volume per one reaction, $\mu\text{l}$		Reagent volumes, $\mu\text{l}$				
		10.00	5.00	0.25	0.50	0.25
Number of clinical samples	Number of analyzed samples*	RT-PCR-mix-1-FL <i>HGV</i>	RT-PCR-mix-2- FEP/FRT	RT-G-mix-2	Polymerase (TaqF)	TM-Revertase (MMIV)
4	8	80	40	2.0	4.0	2.0
6	10	100	50	2.5	5.0	2.5
8	12	120	60	3.0	6.0	3.0
10**	14	140	70	3.5	7.0	3.5
12	16	160	80	4.0	8.0	4.0
14	18	180	90	4.5	9.0	4.5
16	20	200	100	5.0	10.0	5.0
18	22	220	110	5.5	11.0	5.5
20	24	240	120	6.0	12.0	6.0
22	26	260	130	6.5	13.0	6.5
34	38	380	190	9.5	19.0	9.5
46	50	500	250	12.5	25.0	12.5

\* Number of clinical samples + 2 controls of extraction + 2 controls of RT-PCR, (N+4, N – number of clinical samples)

\*\*12-tube panel for extraction

Transfer **15  $\mu\text{l}$**  of the prepared mixture into each tube.

4. Add **10  $\mu\text{l}$**  of **RNA** obtained from clinical samples into the prepared tubes using tips with aerosol barrier.
5. Carry out control amplification reactions:

<b>PCE</b>	–	Add <b>10 µl</b> of <b>RNA sample</b> extracted from the <b>Positive Control HGV-FL-rec</b> sample to the tube labeled PCE (Positive Control of Extraction).
<b>C–</b>	–	Add <b>10 µl</b> of <b>RNA sample</b> extracted from the <b>Negative Control (C–)</b> sample to the tube labeled <b>C–</b> (Negative Control of Extraction).
<b>NCA</b>	–	Add <b>10 µl</b> of <b>buffer for elution</b> to the tube labeled NCA (Negative Control of Amplification).
<b>C+<sub>HGV-FL</sub></b>	–	Add <b>10 µl</b> of <b>Positive Control cDNA HGV-FL</b> to the tube labeled C+HGV-FL (Positive Control of Amplification).

### 8.2.2 Amplification

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

**Table 3**

#### eSens-2 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	Step	Temperature, °C	Time	Cycles
1	50	15 min	1	1	50	15 min	1
	95	15 min		2	95	15 min	1
2	95	5 sec	5	3	95	5 sec	5
	60	20 sec			60	20 sec	
	72	15 sec			72	15 sec	
3	95	5 sec	40	4	95	5 sec	40
	60	20 sec			60	30 sec	
	72	15 sec			72	15 sec	

**NOTE:** Any combination of the tests can be performed in one instrument simultaneously with the use of the unified amplification program (for example, with the tests for *HDV*, *HCV*-genotyping).

**NOTE:** Channels ROX and Cy5 are switched on when necessary (only in MULTIPRIME assays).

Fluorescent signal is detected in the channels for the **FAM**, **JOE**, **ROX** and **Cy5** fluorophores.

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 5FI to 10FI	0.03	On	On	10 %
JOE/Yellow	from 5FI to 10FI	0.03	On	On	10 %

### Test settings for plate-type instruments

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

Channel	Threshold
FAM	The middle of the linear section of the fluorescence intensity increment of the positive control in logarithmic scale.
JOE/HEX	

## 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 the Internal Control cDNA amplification product is detected in the channel for the FAM fluorophore,
- The signal of the *HGV* cDNA 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 RNA sample in the corresponding column of the results grid.

Principle of interpretation is the following:

- The sample is considered **positive** for *HGV* RNA if its *Ct* if the *Ct* value detected in the channel for the JOE fluorophore does not exceed the specified boundary value.
- The sample is considered **negative** for *HGV* RNA if the *Ct* value in the channel for the JOE fluorophore is absent or if the *Ct* value detected in the channel for the JOE fluorophore is greater than the specified boundary value and the *Ct* value in the channel for the FAM fluorophore does not exceed the specified boundary value.
- The sample is considered **equivocal** in case of equivocal result in any channel. The PCR-analysis is recommended to be repeated.

The result of the analysis is considered reliable only if the results obtained for Positive and Negative Controls of amplification as well as for the Positive and Negative Control of extraction are correct (see Table 4 and Table 5).

**Table 4**

**Results for controls**

Control	Stage for control	Ct value in the channel for fluorophore	
		FAM	JOE
C-	RNA extraction, Amplification	< boundary value	absent
PCE	RNA extraction, Amplification	< boundary value	< boundary value
C <sup>+<sub>HGV-FL</sub></sup>	Amplification	< boundary value	< boundary value
NCA	Amplification	absent	absent

**Table 5**

**Boundary Ct values**

Sample	Channel	Boundary Ct values	
		Rotor-Gene Q	CFX96
C+	FAM/Green	27.0	31.0
	YOE/Yellow/HEX	27.0	31.0
C-	FAM/Green	29.0	33.0
PCE	FAM/Green	29.0	33.0
	YOE/Yellow/HEX	27.0	31.0
Clinical samples	FAM/Green	< 29.0	< 33.0
	YOE/Yellow/HEX	< 35.0	< 35.0

## 10 TROUBLESHOOTING

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

1. If the Ct value is absent or it exceeds the specified boundary Ct value for the positive control of extraction (PCE) or the positive control of amplification (C<sup>+<sub>HGV-FL</sub></sup>) in the channel for the JOE fluorophore, the analysis of samples in which HGV RNA was not detected should be repeated starting from the RNA extraction stage.
2. If a Ct value is present for negative controls of extraction (C-) and/or the negative control of amplification (NCA) in the channel for the JOE fluorophore, the analysis of samples in which HGV RNA was detected should be repeated from the RNA extraction stage.

## 11 TRANSPORTATION

eSens HGV 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 HGV QL PCR kit** are to be stored at temperature from minus 24 to minus 16 °C when not in use. All components of the **eSens HGV QL 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:** Positive Control cDNA *HGV-FL*, Positive Control *HGV-FL-rec*, and Internal Control *ICZ-rec* should not be frozen/thawed more than twice. After thawing, Positive Control cDNA *HGV-FL*, Positive Control *HGV-FL-rec*, and Internal Control *ICZ-rec* should be stored at 2–8 °C for up to 6 months.

**NOTE:** RT-PCR-mix-1-FL *HGV* is to be kept away from light.

## 13 SPECIFICATIONS

### 13.1 Sensitivity

The analytical sensitivity of **eSens HGV QL PCR kit** is given in the table below:

**Table 6**

Volume of sample for extraction, µl	RNA/DNA extraction kit	Analytical sensitivity, copies/ml
100	RIBO-prep	500

**NOTE:** The claimed analytical performance characteristics of **eSens HGV QL PCR kit** are guaranteed only when additional reagent kit **RIBO-prep** (manufactured by Federal Budget Institute of Science “Central Research Institute for Epidemiology”) is used.

### 13.2 Specificity

The analytical specificity of **eSens HGV QL PCR kit** is ensured by selection of specific primers and probes as well as by selection as well as stringent reaction conditions. The primers and probes were checked for possible homologies to all sequences deposited in gene banks by sequence comparison analysis as well as by addition of genomic DNA/RNA of the following organisms and viruses into the reaction: *Hepatitis A virus*; *Hepatitis B virus*; *Hepatitis C virus*; *Hepatitis D virus*, *Hepatitis E virus*, *Human immunodeficiency virus*; *Cytomegalovirus*; *Epstein-Barr virus*; *Herpes simplex virus* types 1 and 2; *Enterovirus* (*Coxsackie B1, B2, B3, B4, B5, B6, Polio I, II, III*); *Human rotavirus WA*, *Astrovirus*, *Human herpes virus* types 6 and 8; *Adenovirus* types 2, 3, and 7; and *Homo sapiens*. Cross reactions for marked organisms and viruses are not registered.

The clinical specificity of **eSens HGV QL PCR kit** was confirmed in laboratory clinical trials.

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