



For Professional Use Only

eSens HAV QL PCR kit

REF ES3100B

Instructions for Use

1 INTENDED USE

eSens HAV QL PCR kit is an *in vitro* nucleic acid amplification test for detection of *Hepatitis A virus (HAV)* RNA in clinical material (blood plasma, feces) and environmental objects (concentrated water samples) by 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

PCR analysis includes the following stages: (1) RNA extraction and (2) RNA reverse transcription and cDNA/DNA amplification in the same reaction medium with real-time fluorescence-hybridization detection.

HAV RNA detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific *HAV* primers. In real-time PCR, the amplified product is detected using 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 HAV 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 HAV QL PCR kit uses “hot-start”, which greatly reduces the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by separation of nucleotides and Taq-polymerase by using a chemically modified polymerase (TaqF). Chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

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

Table 1

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

3 CONTENT

eSens HAV QL PCR kit (ES3100B) includes:

Reagent	Description	Volume, ml	Quantity
RT-G-mix-2	colorless clear liquid	0.015	1 tube
RT-PCR-mix-1-FEP/FRT HAV	clear liquid from colorless to light lilac color	0.6	1 tube
RT-PCR-mix-2-FEP/FRT	colorless clear liquid	0.3	1 tube
Polymerase (TaqF)	colorless clear liquid	0.03	1 tube
TM-Revertase (MMiv)	colorless clear liquid	0.015	1 tube
Positive Control cDNA HAV-FL / IC (C ⁺ HAV/IC)*	colorless clear liquid	0.1	1 tube
Negative Control (C-)**	colorless clear liquid	0.5	2 tubes
Positive Control HAV-FL-rec***	colorless clear liquid	0.1	1 tube
Internal Control STI-248-rec (IC)****	colorless clear liquid	0.5	1 tube
RNA-buffer	colorless clear liquid	0.6	1 tube

* this is a complex control for IC and HAV.

** 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).

**** add **10 µl** of **Internal Control STI-248-rec (IC)** during the RNA extraction procedure directly to the sample/lysis mixture.

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

4 ADDITIONAL REQUIREMENTS

- RNA extraction kit.
- 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 rotor for 2 ml reaction tubes.
- PCR box.
- Personal thermocyclers (for example, Rotor-Gene Q (QIAGEN, Germany), CFX 96 Touch, CFX 96 Opus (Bio-Rad, USA), QuantStudio 5 (Thermo Fisher Scientific), or equivalent).
- Personal computer.

- Disposable polypropylene microtubes for PCR (0.1- or 0.2-ml; for example, Axygen, USA).
- 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:

- 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

6.1 Material sampling

eSens HAV QL PCR kit is intended for the analysis of RNA extracted with RNA extraction kits from:

- peripheral blood plasma (serum);
- feces;
- water samples: wastewater concentrates (eluates), drinking water concentrates (eluates);

Container with material must be delivered to laboratory in a tank with ice within 24 h.

Preparation of the samples

6.1.1 Peripheral blood plasma (serum).

Blood sampling must be carrying out in the morning on an empty stomach. To obtain plasma, mix the blood with 3 % EDTA in a tube (20:1, v/v). Close the tube and turn it upside down and back several times. Centrifuge the tube at 800-1600 g for 20 min and transfer the plasma to a new tube within 6 h after

taking blood. To obtain serum, tubes with blood should be kept at room temperature until a clot forms completely. Centrifuge the tube at 800-1600 g for 10 min at room temperature and then transfer the serum to a new tube. Material can be stored at 2–8 °C for 3 days and at ≤ – 68 °C for a long time.

6.1.2 Feces.

Prepare a clarified fecal extract. For preparation use liquid stool consistency, fresh fecal suspension, or frozen fecal suspension with glycerol. Homogenize fecal suspension on the vortex. Centrifuge the suspension at 10.000 g for 5 min at room temperature. Use the supernatant for RNA extraction. If necessary, store the supernatant in a new tube. The material can be stored at 2–8 °C for 1 day and at ≤ – 68 °C for a long time.

NOTE: Only one freeze–thaw cycle of clinical material is allowed.

NOTE: For preparation of fecal suspension: 1. Add 0.8 ml of PBS (or sterile isotonic NaCl solution) to 1.5-ml microcentrifuge tubes. 2. Using tips with aerosol barrier, add 0.1 g of feces and thoroughly resuspend on vortex until a homogeneous suspension forms. If the fecal consistency is liquid, steps 1 and 2 are not required.

For a long storage of suspension, add glycerol to 15 % final concentration, mix thoroughly, incubate the suspension at room temperature for 1 h, and then freeze.

6.1.3 Concentrated water samples (eluates).

Material is used for RNA extraction without pretreatment. If the sample contains visible admixtures or has a visible color, vortex tubes with sample and then centrifuge at 10.000 g for 1 min at room temperature. Use the supernatant for RNA extraction. The material can be stored at 2–8 °C for 1 day and at ≤ – 68 °C for a long time.

NOTE: Only one freeze–thaw cycle of clinical material is allowed.

7 WORKING CONDITIONS

eSens HAV 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.**

Add **50 µl** of **Negative Control** to each tube if using fecal samples.

8.2 Preparing PCR

It is recommended to carry out reverse transcription combined with PCR amplification (RT-PCR) within 30 min after RNA extraction.

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

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.

NOTE: Mix the reaction mixture components just before the analysis. Mix reagents for the required number of reactions for test and control samples according to **Table 2**. Carry out 2 control amplification reactions even while testing only one RNA sample. It is recommended to mix the reagents for even number of reactions for more precise reagents dosing.

1. Thaw the reagents and vortex the tubes thoroughly and sediment drops from walls of tubes.
2. Prepare the required number of tubes including controls.
3. Mix **RT-PCR-mix-1-FEP/FRT HAV** with **RT-PCR-mix-1-FEP/FRT**, **RT-G-mix-2**, **polymerase (TaqF)**, and **TM-Revertase (MMIv)** according to **Table 2**. Vortex the tubes thoroughly and sediment drops from walls of tubes.

Table 2

Scheme of reaction mixture preparation						
Total reaction volume is 25 µl						
Reagent volume per one reaction is 15 µl						
RNA sample volume is 10 µl						
Reagent volume per 1 reaction (µl)		10.00	5.00	0.25	0.50	0.25
Number of clinical samples*	Number of reactions**	RT-PCR-mix-1-FEP/FRT HAV	RT-PCR-mix-2-FEP/FRT	RT-G-mix-2	Polymerase (TaqF)	TM-Revertase (MMIv)
4	6	60	30	1.5	3.0	1.5
6	8	80	40	2.0	4.0	2.0
8	10	100	50	2.5	5.0	2.5
10	12	120	60	3.0	6.0	3.0
12	14	140	70	3.5	7.0	3.5
14	16	160	80	4.0	8.0	4.0
16	18	180	90	4.5	9.0	4.5
18	20	200	100	5.0	10.0	5.0
20	22	220	110	5.5	11.0	5.5
22	24	240	120	6.0	12.0	6.0
24	26	260	130	6.5	13.0	6.5
26	28	280	140	7.0	14.0	7.0
28	30	300	150	7.5	15.0	7.5
30	32	320	160	8.0	16.0	8.0
32	34	340	170	8.5	17.0	8.5
34	36	360	180	9.0	18.0	9.0
36	38	380	190	9.5	19.0	9.5
38	40	400	200	10.0	20.0	10.0
40	42	420	210	10.5	21.0	10.5
42	44	440	220	11.0	22.0	11.0
44	46	460	230	11.5	23.0	11.5
46	48	480	240	12.0	24.0	12.0
48	50	500	250	12.5	25.0	12.5
50	52	520	260	13.0	26.0	13.0
52	54	540	270	13.5	27.0	13.5

* The number of clinical samples (**N**) and two controls of extraction (C- and PCE).

** The number of clinical samples (**N**), two controls of extraction (C- and PCE), and two controls of amplification (C+ HAV / IC and NCA).

4. Transfer **15 µl** of the prepared mixture to the tubes.

5. Add **10 µl** of **RNA** obtained from clinical or control samples into the prepared tubes using tips with aerosol barrier. Carefully mix by pipetting.
6. Carry out the control amplification reactions:

NCA	– Add 10 µl of RNA-buffer to the tube labeled NCA (Negative Control of Amplification).
C+_{HAV/IC}	– Add 10 µl of Positive Control cDNA HAV-FL / IC (C+_{HAV/IC}) to the tube labeled C+ _{HAV/IC} (Positive Control of Amplification).

8.2.2 Reverse transcription and amplification

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

Table 3

eSens-3 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	30 min	1	1	50	30 min	1
	95	15 min			95	15 min	
2	95	5 s	5	2	95	5 s	2
	60	20 s			60	20 s	
	72	15 s			72	15 s	
3	95	5 s	40	3	95	5 s	3
	60	20 s			50	20 s	
	72	15 s			95	15 s	

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

2. Insert tubes into the instrument.
3. Adjust the fluorescence channel sensitivity and carry out data analysis.

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

Test settings for plate-type instruments

Channel	Threshold fluorescence
FAM	In Threshold fluorescence menu, set the threshold line at a level where the fluorescence curves are linear. It is recommended to select the threshold line level equal to 200 for FAM channel and 500 for JOE/HEX channel. Normally, the threshold line should cross only the sigmoid curves of signal accumulation of positive samples and controls and should not cross the baseline; otherwise, the threshold level should be raised.
JOE/HEX	

9 DATA ANALYSIS

IC is detected in the FAM/Green fluorescence channel, HAV RNA is detected in the JOE/Yellow/HEX fluorescence channel.

Results interpretation for samples

The results are interpreted by the software of instrument by the crossing (or not-crossing) of the fluorescence curve with the threshold line.

Principle of interpretation:

- HAV RNA is **detected** in a sample if *Ct* of a sample does not exceed the specified boundary value in the JOE/Yellow/HEX channel. Moreover, the fluorescence curve should cross the threshold line in the area of exponential fluorescence growth.
- HAV RNA is **not detected** in a sample if its *Ct* is not defined in the result grid in the JOE/Yellow/HEX channel (the fluorescence curve does not cross the threshold line) while *Ct* in the FAM/Green channel does not exceed the specified boundary value.
- The result is considered to be **invalid** if *Ct* of a sample in the FAM/Green channel is absent whereas *Ct* in the JOE/Yellow/HEX channel is either absent or greater than the specified boundary value. It is necessary to repeat RNA extraction for such a sample.
- The result is considered to be **equivocal** if *Ct* of a sample exceeds the specified boundary value in the JOE/Yellow/HEX channel. It is necessary to repeat RNA extraction for such a sample. If the result repeats as positive, the sample is considered to be positive. If the result repeats as negative, the sample is considered to be equivocal.

Results interpretation for control samples

The result of the analysis is considered reliable only if the results obtained for both positive and negative controls of amplification as well as for the positive and negative controls of extraction are correct (see Table 4).

Table 4

Results for controls

Sample	Rotor-type instrument		Plate-type instrument	
	Channel for fluorophore			
	FAM	JOE	FAM	JOE
NCA, IC	31	-	35	-
PCE	31	28	35	28
C-	-	-	-	-
C+/IC	31	27	33	29

10 TROUBLESHOOTING

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

1. If the Ct value determined for the Positive Control of Extraction (PCE) in the JOE/Yellow/HEX channel exceed the boundary value or absent, the PCR analysis (beginning with the RNA extraction stage) should be repeated for all samples in which HAV RNA was not detected.
2. If the Ct value determined for the Positive Control of Amplification (C+) in the JOE/Yellow/HEX channel exceed the boundary value or absent, the PCR analysis (beginning with the RT-PCR stage) should be repeated for all samples in which HAV RNA was not detected.
3. If the positive signal is detected for the Negative Control of Amplification (NCA) and/or Negative Control of Extraction (C-) in the JOE/Yellow/HEX channel, the PCR analysis (beginning with the RNA extraction stage) should be repeated for all samples in which HAV RNA was detected.

11 TRANSPORTATION

eSens HAV 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 HAV QL PCR kit** (except for RT-G-mix-2, polymerase (TaqF), TM-Revertase (MMIv), RT-PCR-mix-1-FEP/FRT HAV, and RT-PCR-mix-2-FEP/FRT) are to be stored at 2–8 °C when not in use. All components of the **eSens HAV QL PCR kit** are stable until the expiration date on the label. The shelf life of opened reagents is the same as that of unopened reagents, unless otherwise stated.

NOTE: RT-G-mix-2, polymerase (TaqF), TM-Revertase (MMIv), RT-PCR-mix-1-FEP/FRT HAV, and RT-PCR-mix-2-FEP/FRT are to be stored at the temperature from minus 24 to minus 16 °C
RT-PCR-mix-1-FEP/ FRT HAV is to be kept away from light.

13 SPECIFICATIONS

13.1 Sensitivity

Variant	Volume, μ l	Nucleic extraction kit	Material	Sensitivity, copies/ml
ES3100B	100	RIBO-prep	Blood plasma (serum), clarified fecal extracts, concentrated water samples (eluates)	500
	100	ePure Viral Nucleic Acid Extraction Kit	Blood plasma (serum), concentrated water samples (eluates)	500
	200	ePure Viral Nucleic Acid Extraction Kit	Blood plasma (serum), concentrated water samples (eluates)	250
	1000	ePure Viral Nucleic Acid Extraction Kit	Blood plasma (serum), concentrated water samples (eluates)	50

13.2 Specificity

The analytical specificity of **eSens HAV QL PCR kit** is ensured by selection of specific primers and probes as well as strict reaction conditions. The primers and probes were checked for possible homologies to all sequences published in gene banks by sequence comparison analysis. The analytical specificity of **eSens HAV QL PCR kit** was checked by testing RNA/DNA of the following organisms and viruses: *HBV*, *HCV*, *HDV*, *HEV*, *HGV*, *HIV*, *CMV*, *EBV*, *HSV I* and *II* types, *HSV VI* and *VIII* types, *Enterovirus* (Coxsackie B1, B2, B3, B4, B5, B6, Polio I, II, III), human *Rotavirus* WA, *Astrovirus*, *Norovirus I* and *II* types, *Adenovirus* (types II, III, VII), *Shigella*, *Salmonella*, *Yersinia*, *Campylobacter*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Homo sapiens*.














Cross-reactions for the listed organisms were not detected.

The clinical specificity of **eSens HAV 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		Caution
 LOT	Batch code		Contains sufficient for <n> tests
 IVD	<i>In vitro</i> diagnostic medical device		Use-by Date
 VER	Version		Consult instructions for use
	Temperature limit		Keep away from sunlight
	Manufacturer	NCA	Negative control of amplification
	Date of manufacture	C-	Negative control of extraction
 EC REP	Authorized representative in the European Community	C+_{HAV}/IC	Positive control of amplification
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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