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** |
|---|---|---|
| cDNA-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 (MMlv)** | 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)

  If using RIBO-prep kit, extract the RNA according to the manufacturer's protocol taking into account next additions and improvements:

  - The volume of plasma (serum) or concentrated water samples (eluates) is 100 µl, the volume of feces samples is 50 µl.
  - Add 50 µl of Negative Control to each tube if using fecal samples

- For the automatic extraction

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

In the extraction procedure it is necessary to carry out the control reaction:

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 *HAV*-FL-rec to the tube labeled PCE (Positive Control of Extraction).

**NOTE:** Use only disposable sterile plastic materials with “RNase-free” and ”DNase-free” marking for the work with RNA.

## 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 (MMlv)** 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<br>Reagent volume per one reaction is 15 µl<br>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 (MMlv)** |
| **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** | **5** |
| | **60** | **20 s** | | | **60** | **20 s** | |
| | **72** | **15 s** | | | **72** | **15 s** | |
| **3** | **95** | **5 s** | **40** | **3** | **95** | **5 s** | **40** |
| | **60** | **20 s*** | | | **60** | **30 s*** | |
| | **72** | **15 s** | | | **72** | **15 s** | |

*** Fluorescent signal is detected** in the channels for the **FAM** and **JOE/HEX** 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 Fl to 10 Fl | 0.03 | on | on | 10 % |
| JOE/Yellow | from 5 Fl to 10 Fl | 0.03 | on | on | 10 % |

**Test settings for plate-type instruments**

| Channel | Threshold fluorescence |
|---|---|
| FAM<br>JOE/HEX | 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. |

# 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**

| Control | Stage for control | Ct value in channel JOE/Yellow/HEX | Ct value in channel FAM/Green |
|---|---|---|---|
| **C-** | RNA extraction | Absent | ≤ B1 boundary value |
| **PCE** | RNA extraction | ≤ K1 boundary value | ≤ B1 boundary value |
| **NCA** | RT-PCR | Absent | Absent |
| **C+ HAV / IC** | RT-PCR | ≤ K2 boundary value | ≤ K2 boundary value |

**Table 5**

**Boundary *Ct* values**

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

**HAV RNA is detected in a sample** if the *Ct* value detected in the channel for the **JOE** fluorophore is less than 35.

**HAV RNA is not detected in a sample** if the *Ct* value in the channel for the **JOE** fluorophore is absent while the *Ct* value detected in the channel for the **FAM** fluorophore for IC is less than the *Ct* value B1 specified.

## 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 (MMlv), 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 (MMlv), 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, µl | 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* (Coxsakie 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

| Symbol | Meaning | Symbol | Meaning |
|---|---|---|---|
| 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 | **$C+_{HAV/IC}$** | 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 |
|---|---|---|
| 02_01/2025 | 8.2.2. Amplification | Specification of the fluorescence reading point. |
| 03_05/2025 | 9 DATA ANALYSIS | Table 4 was added |
| 04_06/2026 | Table 1 | cDNA-target |
| | Section 8.1 RNA extraction | Multiple modifications in the procedure |
| | Table 5 | Correction of C- and NCA values |

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