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For Professional Use Only

# AmpliSens® *HBV*-genotype-FRT

## PCR kit

## Instruction Manual

**AmpliSens®**

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## 1. INTENDED USE

**AmpliSens® *HBV*-genotype-FRT** PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection and differentiation of *hepatitis B virus* (*HBV*) genotypes A, B, C and D in the clinical material (blood plasma) using real-time hybridization-fluorescence detection of amplified products.

**AmpliSens® *HBV*-genotype-FRT** PCR kit is recommended to use after detection of *hepatitis B virus* DNA by PCR kits for qualitative or quantitative analysis manufactured by Federal Budget Institute of Science "Central Research Institute for Epidemiology" (for example, **AmpliSens® *HBV*-FRT** or **AmpliSens® *HBV*-Monitor-FRT)** with the use of hybridization-fluorescence detection.

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

## 2. PRINCIPLE OF PCR DETECTION

The principle of analysis is based on the DNA extraction from blood plasma and DNA amplification with real-time hybridization-fluorescence detection. The samples of DNA extracted from the clinical material, in which the positive results were obtained at the stage of qualitative and/or quantitative detection, can be used.

Detection of *HBV* genotypes A, B, C and D by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific 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.

**AmpliSens® *HBV*-genotype-FRT** 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.

Detection of *HBV* genotypes A, B, C and D is carried out in a single tube. The PCR kit is designed for the PCR instruments with four and more fluorescence detection channels. The channels for detection of *HBV* genotypes are specified in the table 1.

Table 1

| *Channel for the fluorophore* | *HBV genotype* |
|---|---|
| **FAM** | **C** |
| **JOE** | **D** |
| **ROX** | **B** |
| **Cy5** | **A** |

## 3. CONTENT

**AmpliSens® *HBV*-genotype-FRT** PCR kit is produced in 1 form:

**AmpliSens® *HBV*-genotype-FRT** PCR kit variant FRT-50 F, **REF** R-V5-G-F-CE.

**AmpliSens® *HBV*-genotype-FRT PCR kit variant FRT-50 F** includes:

| *Reagent* | *Description* | *Volume, ml* | *Quantity* |
|---|---|---|---|
| **PCR-mix-1-FRT *HBV* genotypes C/D/B/A** | colorless clear liquid | 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 |
| **Positive Control DNA *HBV* genotypes B/A ($C+_{B/A}$)** | colorless clear liquid | 0.2 | 1 tube |
| **Positive Control DNA *HBV* genotypes C/D ($C+_{C/D}$)** | colorless clear liquid | 0.2 | 1 tube |
| **TE-buffer** | colorless clear liquid | 0.07 | 1 tube |
| **Negative Control (C–)*** | colorless clear liquid | 1.2 | 1 tube |

* must be used in the extraction procedure as Negative Control of Extraction (see RIBO-prep, **REF** K2-9-Et-50-CE protocol).

**AmpliSens® *HBV*-genotype-FRT** PCR kit is intended for 55 reactions (including controls).

## 4. ADDITIONAL REQUIREMENTS

- DNA extraction kit.
- Disposable powder-free gloves and a laboratory coat.
- Pipettes (adjustable).
- Sterile RNase-free 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 3000/6000 (Corbett Research, Australia); Rotor-Gene Q (QIAGEN, Germany) iCycler iQ5 (Bio-Rad, USA); CFX 96 (Bio-Rad, USA)).
- Disposable polypropylene PCR tubes (0.1- or 0.2-ml):
  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 or strips of four 0.1-ml Rotor-Gene PCR tubes if a rotor-type instrument is used.
- Refrigerator for 2–8 °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 barriers 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 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 immediately.
- 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

Obtaining samples of biological materials for PCR-analysis, transportation, and storage are described in the manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

**AmpliSens® *HBV*-genotype-FRT** PCR kit is intended for analysis of the DNA extracted with DNA extraction kits from the clinical material (peripheral blood plasma).

Collect a blood sample in a tube with 3 % EDTA solution in the ratio 20:1 (20 parts of blood for 1 part of EDTA). Invert the closed tube several times to ensure adequate mixing. Remove and transfer the plasma specimen in a new tube within 6 h from the time of blood taking. To do this, centrifuge the tube with blood at 800 – 1,600 g for 20 min. Remove plasma and transfer in in a new disposable tube. Plasma can be stored at 2–8 °C for up to 3 days and at no more than minus 68 °C for a long time.

## 7. WORKING CONDITIONS

**AmpliSens® *HBV*-genotype-FRT** PCR kit should be used at 18–25 °C.

## 8. PROTOCOL

### 8.1. DNA extraction

It is recommended to use the following nucleic acid extraction kits:

– RIBO-prep, **REF** K2-9-Et-50-CE.

Extract the DNA according to the manufacturer's protocol.

### 8.3. Preparing PCR

#### 8.3.1 Preparing tubes for PCR

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

All components of the reaction mix should be mixed immediately before use.

1. Before starting work, thaw the all reagents of the kit, sediment the drops by short

centrifugation (1-2 s).

2. Take the required number of tubes/strips for amplification of the DNA obtained from clinical and control samples (one control of extraction, three controls of amplification).
3. For reaction mixture preparation, add to a new tube the following reagents (calculating per one reaction): **10 µl** of **PCR-mix-1-FRT *HBV* genotypes C/D/B/A**, **5 µl** of **RT-PCR-mix-2-FEP/FRT** and **0.5 µl** of **polymerase (TaqF)** (see also table 2).

Table 2

**Scheme of reaction mixture preparation**

| | | Reagent volume for specified number of reactions | | |
|---|---|---|---|---|
| **Reagent volume per one reaction, µl** | | **10.0** | **5.0** | **0.5** |
| **Number of clinical samples** | **Number of reactions [1]** | **PCR-mix-1-FRT[2]** | **RT-PCR-mix-2-FEP/FRT[2]** | **Polymerase (TaqF)[2]** |
| **4** | **8** | **90** | **45** | **4.5** |
| **5** | **9** | **100** | **50** | **5.0** |
| **6** | **10** | **110** | **55** | **5.5** |
| **7** | **11** | **120** | **60** | **6.0** |
| **8** | **12** | **130** | **65** | **6.5** |
| **9** | **13** | **140** | **70** | **7.0** |
| **10** | **14** | **150** | **75** | **7.5** |
| **11** | **15** | **160** | **80** | **8.0** |
| **12** | **16** | **170** | **85** | **8.5** |

4. Mix the prepared reaction mixture thoroughly by vortexing and sediment the drops by short centrifugation.
5. Add into each tube for amplification **15 µl** of prepared mixture.
6. Add **10 µl** of **DNA samples** obtained at the DNA extraction stage to prepared tubes.
7. Carry out the control reactions:

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

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

**$C+_{B/A}$** - Add **10 µl** of **Positive Control DNA *HBV* genotypes B/A ($C+_{B/A}$)** to the tube labeled $C+_{B/A}$ (Positive Control of Amplification).

[1] Number of clinical samples + 1 control of extraction + 3 controls of amplification (N+4, N is number of clinical samples).
[2] The reagent volumes are specified with a reserve for 1 extra reaction.

**$C+_{C/D}$** - Add **10 µl** of **Positive Control DNA *HBV* genotypes C/D ($C+_{C/D}$)** to the tube labeled $C+_{C/D}$ (Positive Control of Amplification).

### 8.3.2. Amplification

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

Table 3

**AmpliSens 1 amplification program**

| | ***Rotor-type instruments***[3] | | | ***Plate-type instruments***[4] | | |
|---|---|---|---|---|---|---|
| ***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 acquiring | | **60** | 30 s Fluorescence acquiring | |
| | **72** | 15 s | | **72** | 15 s | |

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

2. Adjust the fluorescence channel sensitivity according to the *Important Product Information Bulletin* and Guidelines [2].
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.

## 9. DATA ANALYSIS

Analysis of results is performed by the software of the real-time PCR instrument used by measuring fluorescence signal accumulation in four channels. The channels for detection of *HBV* genotypes are specified in the table 4.

[3] For example, Rotor-Gene 3000, Rotor-Gene 6000 (Corbett Research, Australia), Rotor-Gene Q (QIAGEN, Germany).
[4] For example, iCycler iQ5 (Bio-Rad, USA), CFX96 (Bio-Rad, USA).

Table 4

| *Channel for the fluorophore* | *HBV genotype* |
|---|---|
| **FAM** | **C** |
| **JOE** | **D** |
| **ROX** | **B** |
| **Cy5** | **A** |

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 cDNA sample in the corresponding column of the results grid.

The result of amplification in the channel is considered *positive* if the fluorescence curve crosses the threshold line in the area of reliable growth of fluorescence. The result is considered *negative* if the fluorescence curve does not cross the threshold line (*Ct* or *Cp* value is absent). The result is considered *equivocal* in all other cases.

**Interpretation of results for control samples**

**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 Negative Control of extraction are correct (seeTable 5).**

Table 5

**Results for controls**

| **Control** | **Stage for control** | ***Ct* value in the channel for fluorophore** | | | |
|---|---|---|---|---|---|
| | | **FAM** | **JOE** | **ROX** | **Cy5** |
| C– | DNA extraction | Absent | Absent | Absent | Absent |
| NCA | PCR | Absent | Absent | Absent | Absent |
| **$C+_{B/A}$** | PCR | Absent | Absent | **<** boundary value | **<** boundary value |
| **$C+_{C/D}$** | PCR | **<** boundary value | **<** boundary value | Absent | Absent |

Boundary *Ct* values are specified in the *Important Product Information Bulletin* enclosed to the PCR kit. See also Guidelines [2]

**Interpretation of results for clinical samples**

1. If the *Ct* value is detected for the sample only in the channel for the **FAM** fluorophore, then the result is **"*HBV* genotype C"**.
2. If the *Ct* value is detected for the sample only in the channel for the **JOE** fluorophore,

then the result is **"*HBV* genotype D"**.

3. If the *Ct* value is detected for the sample only in the channel for the **ROX** fluorophore, then the result is **"*HBV* genotype B"**.
4. If the *Ct* value is detected for the sample only in the channel for the **Cy5** fluorophore, then the result is **"*HBV* genotype A"**.
5. If two or more *Ct* values are detected for the sample, then the result with double, triple and etc genotype is given.
6. If the *Ct* value is not detected for the sample, then the result is **"*HBV* genotype is not detected"**. If it is known that the *HBV* DNA concentration in this sample is in the range of reagent kit analytical sensitivity, then the result is **"*HBV* genotype is not detected due to the low viral load"**.

## 10. TROUBLESHOOTING

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

1. If the *Ct* value even for one of the Positive Control of Amplification ($C+_{B/A}$ or $C+_{C/D}$) exceeds the boundary *Ct* value specified in the *Important Product Information Bulletin* or is absent, the PCR analysis should be repeated for all negative samples (beginning with the amplification stage).
2. If the positive signal is detected for the Negative Control of Extraction (C–) and/or Negative Control of Amplification (NCA) in any channel, the PCR analysis should be repeated for all positive samples (beginning with the amplification stage).

## 11. TRANSPORTATION

**AmpliSens® *HBV*-genotype-FRT** PCR kit should be transported at 2–8 °C for no longer than 5 days.

## 12. STABILITY AND STORAGE

All components of the **AmpliSens® *HBV*-genotype-FRT** PCR kit are to be stored at the temperature from minus 24 to minus 16 °C when not in use. All components of the **AmpliSens® *HBV*-genotype-FRT** PCR kit are stable until the expiry date stated on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

PCR-mix-1-FRT *HBV* genotypes C/D/B/A is to be kept away from light.

## 13. SPECIFICATIONS

### 13.1. Sensitivity

| Clinical material | Extraction value, µl | Nucleic acid extraction kit | Sensitivity, IU/ml |
|---|---|---|---|
| Blood plasma | 100 | RIBO-prep | 500 |

### 13.2. Specificity

The analytical specificity of **AmpliSens® *HBV*-genotype-FRT** PCR kit is ensured by the selection of specific primers and probes as well as stringent reaction conditions. The primers and probes have been checked for possible homologies to all sequences published in gene banks by sequence comparison analysis.

The analytical sensitivity assessment has shown the absence of cross-reactions between *HBV* genotypes A, B, C, D, E, F, G and H with the use of highly concentrated recombinant positive control samples and blood plasma samples of respective *HBV* genotypes.

The clinical specificity of **AmpliSens® *HBV*-genotype-FRT** PCR kit was confirmed in laboratory clinical trials.

## 14. REFERENCES


1. Handbook “Sampling, Transportation, and Storage of Clinical Material for PCR diagnostics”, developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow, 2010.
2. Guidelines to the **AmpliSens® *HBV*-genotype-FRT** PCR kit for qualitative detection and differentiation of *hepatitis B virus* (*HBV*) genotypes A, B, C and D in the clinical material (blood plasma) by the polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology”.


## 15. QUALITY CONTROL

In compliance with Federal Budget Institute of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Quality Management System, each lot of the **AmpliSens® *HBV*-genotype-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

## 16. KEY TO SYMBOLS USED

| | | | |
|---|---|---|---|
| REF | Catalogue number |  | Caution |
| LOT | Batch code |  | Sufficient for |
| RUO | Research use only |  | Expiration Date |
| VER | Version |  | Consult instructions for use |
|  | Temperature limitation |  | Keep away from sunlight |
|  | Manufacturer | **NCA** | Negative control of amplification |
|  | Date of manufacture | **C–** | Negative control of extraction |
| | | **C+** | Positive control of amplification |