RUO

For Professional Use Only

AmpliSens® *HBV*-FEP
PCR kit

Instruction Manual

AmpliSens®

Federal Budget Institute of
Science “Central Research
Institute for Epidemiology”
3A Novogireevskaya Street
Moscow 111123 Russia

TABLE OF CONTENTS

1. INTENDED USE 3
2. PRINCIPLE OF PCR DETECTION 3
3. CONTENT 3
4. ADDITIONAL REQUIREMENTS 4
5. GENERAL PRECAUTIONS 5
6. SAMPLING AND HANDLING 6
7. WORKING CONDITIONS 6
8. PROTOCOL 6
9. DATA ANALYSIS 9
10. TROUBLESHOOTING 10
11. TRANSPORTATION 11
12. STABILITY AND STORAGE 11
13. SPECIFICATIONS 11
14. REFERENCES 12
15. QUALITY CONTROL 12
16. KEY TO SYMBOLS USED 13

1. INTENDED USE

AmpliSens® ***HBV*-FEP** PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of hepatitis B virus (*HBV*) DNA in the clinical materials (blood plasma) by means of end-point hybridization-fluorescence detection of amplified products.

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

2. PRINCIPLE OF PCR DETECTION

Hepatitis B virus (*HBV*) DNA is isolated from blood plasma together with internal control sample (IC). *HBV* detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using special HBV primers. In end-point PCR the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes which bind specifically to the amplified product. Multichannel rotor-type fluorometer is specially designed to detect fluorescent excitation from the fluorophores in a reaction mix after PCR. **F**luorescent **E**nd-**P**oint PCR (FEP-PCR) allows the detection of accumulating product without re-opening of the reaction tubes after the PCR run. **AmpliSens® *HBV*-FEP** 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 chemically modified polymerase (TaqF). Chemically modified polymerase (TaqF) is activated by heating at 95ºC for 15 min.

The IC amplification product is detected in the FAM channel. The *HBV* cDNA amplification product is detected in the JOE/HEX channel. The Positive Control of Extraction, Positive Control-1-*HBV,* is detected in FAM (IC) and JOE/HEX (*HBV*) channels. DNA calibrator PIC2 *HBV* is a complex control for *HBV* and IC. It is detected in FAM (IC) and JOE/HEX (*HBV*) channels.

3. CONTENT

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

AmpliSens® *HBV*-FEP PCR kit, **REF** V5-FEP-CE.

AmpliSens® *HBV*-FEP PCR kit includes:

Reagent	***Description***	***Volume, ml***	***Quantity***
PCR-mix-1-FL *HBV*	colorless clear liquid	0.3	4 tubes
PCR-mix-2-FRT	colorless clear liquid	0.2	4 tubes
Polymerase (TaqF)	colorless clear liquid	0.02	4 tubes
Mineral oil for PCR	colorless viscous liquid	4.0	1 bottle
DNA calibrator PIC2 *HBV**	colorless clear liquid	0.1	4 tubes
Buffer for elution	colorless clear liquid	1.2	2 tubes
Negative Control (C–)**	colorless clear liquid	1.2	4 tubes
Positive Control-1-*HBV****	colorless clear liquid	0.06	4 tubes
Internal Control STI-87 (IC)****	colorless clear liquid	0.28	4 tubes

* Serves as a Positive Control of Amplification (C+).

** Must be used in the isolation procedure as Negative Control of Extraction.

*** Must be used in the isolation procedure as Positive Control of Extraction.

**** Must be added during the RNA/DNA extraction procedure directly to the sample/lysis mixture.

AmpliSens® *HBV*-FEP PCR kit is intended for 112 amplification reactions, including controls.

4. ADDITIONAL REQUIREMENTS

- DNA isolation kit
- 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
- Personal thermal cyclers (for example, Gradient Palm Cycler (Corbett Research, Australia), GeneAmp PCR System 2700 or GeneAmp PCR System 2400 (Applied Biosystems, USA), Uno-2 (Biometra, Germany), MiniCycler PTC-100 (MJ Research,

USA), Terzik (DNA-Technology, Russia) or equivalent instrument)

- Fluorometer ALA-1/4 (Biosan, Latvia) or equivalent instrument
- Disposable polypropylene microtubes for PCR with 0.5 (0.2) ml capacity (for example, Axygen, USA)
- Refrigerator for 2–8 °C
- Deep-freezer with temperature below or at minus 16 °C
- Waste bin for used tips

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers and use new tip for every procedure.
- Store extracted positive material (samples, controls and amplicons) away from all other reagents and add it to the reaction mix in a separate area.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats, 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 all samples and unused reagents in compliance with local authorities' requirements.
- Samples should be considered potentially infectious and handled in a biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant such as 0.5 % sodium hypochlorite, or other suitable disinfectant.
- Avoid contact with the skin, eyes and mucous membranes. If skin, eyes and mucous membranes contact, immediately flush with water and seek medical attention
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- The laboratory process must be one-directional; it should begin in the Extraction Area and then move to the Amplification and Detection Areas. Do not return samples, equipment and reagents to the area in which 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 detail in the manufacture's handbook [1]. It is recommended that this handbook is read before starting the work.

AmpliSens® *HBV*-FEP PCR kit is intended to analyze DNA extracted with a DNA isolation kits from

- *Peripheral blood plasma*.

Blood samples are taken after overnight fasting into tubes with 3% EDTA solution (1 : 20). 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. Blood plasma can be stored unfrozen (at 2–8 °C) for at most 3 days or frozen (at or below 68 °C) for a long time. In some cases, blood serum can be used. In this case, the analytical sensitivity of the reagent kit is retained; however, the clinical sensitivity may be significantly decreased as a result of precipitation of viral particles during blood clot retraction. Blood serum can be stored unfrozen (at 2–8 °C) for at most 3 days or frozen (at or below 68 °C) for a long time.

7. WORKING CONDITIONS

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

8. PROTOCOL

8.1. DNA Isolation

It’s recommended that the following nucleic acid extraction kits are used:

- RIBO-sorb, REF K2-1-Et-100-CE
- RIBO-prep, REF K2-9-Et-100-CE
- MAGNO-sorb, REF K2-16-1000-CE
- Automatic device NucliSENS easyMAG can also be used.

Carry out the RNA/DNA isolation according to the manual provided by the manufacturer.

For Positive Control of Extraction (PCE), mix **10 µl** of **Positive Control-1-*HBV*** and **90 µl of Negative Control**.

Volume of **Internal Control STI-87** that must be added at the extraction stage depends on the RNA/DNA isolation kit used:

- **10 µl** in the case of RIBO-sorb, REF K2-1-Et-100-CE
- **10 µl** in the case of RIBO-prep, REF K2-9-Et-100-CE
- **0.28 ml** in the case of MAGNO-sorb, REF K2-16-1000-CE if a 24-tube panel is used. For other panels, see the Manufacturer's protocol for this isolation kit.

- If using RIBO-sorb kit, after addition of clinical and control samples to lysis solution warm the mixture at 60 for 10 min prior to sorbent addition.

If using RIBO-prep kit:

- after preparing the Controls, incubate tubes with them at **65 °C for 5 min** and then vortex. Make sure there are no drops on the walls of the tubes.
- after adding **Solution for Precipitation**, **Washing Solution 3**, **Washing Solution 4**, and **RNA-buffer**, centrifuge all tubes at 12,000 g.

If using the MAGNO-sorb kit:

- to extract DNA from blood plasma sample of 1000 µl, the volume of the **Internal Control STI-rec** required for extraction from 24 samples is **0.28 ml**.
- to prepare the Positive Control of extraction, **PCE**, add **90 µl** of the **Negative Control (C–)** sample and **10 µl** of the **Positive Control-1-*HBV*** sample to a tube containing **lysis solution**.
- to prepare the Negative Control of extraction, **C–**, add **100 µl** of the **Negative Control (C–)** sample to a tube containing **lysis solution**.
- the volume of **Buffer for elution** required for extraction from both 1000 and 200 µl of blood plasma samples is **70 µl**.

If using NucliSENS easyMAG automated system

- "EM-plus" kit REF K2-15-96-CE (manufactured by FBIS CRIE) must be used
- set a sample volume as 0.1 ml or 1 ml
- set the eluate volume as 50-60 µl (up to 100 µl)
- both *On-board* and *Off-board* Lysis Buffer Dispensing and Lysis Incubation modes can be used
  For details, see the Guidelines.

The purified DNA can be stored at 2–8 °C for one week and at temperatures not higher than minus 16 °C for one year.

8.2. Preparing the PCR

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

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 Appendix 1.

8.2.1 Preparing tubes for PCR

1. 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 0.2- or 0.5-ml tubes for amplification for the clinical and

control samples (two controls of extraction and one control of amplification) and the **Background** samples. The type of tubes depends on the PCR instrument used for analysis.

3. To prepare the reaction mix, if **Background** samples are prepared, mix reagents (**10 µl of PCR-mix-1-FL *HBV*** and **5 µl of PCR-mix-2-FRT** per one reaction) in a new sterile tube (see also Appendix 1, Part A). Thoroughly vortex the mixture, make sure that there are no drops on the caps of the tubes.
4. Mark two tubes as **Background** and add 15 µl of the prepared mixture (without **Polymerase TaqF**) and 10 µl of the **buffer for elution** to each tube. Mix by pipetting. Add above **1** drop of **mineral oil for PCR**.

Background samples can be stored at 2–20 ºC for 1month and used many times. **Background** samples can be used many times only with the PCR kit of the same lot, the same type of isolation reagents, and the same type of amplification tubes are used. Keep away from light.

5. Add **Polymerase TaqF** (**0.5 µl** per sample) to the remaining reaction mixture (see also Appendix 1, Part A). Thoroughly vortex the mixture, make sure that there are no drops on the caps of the tubes.

Volumes of **Polymerase TaqF** added to the reaction mixture are calculated after deduction of the enzyme volume (30 µl) withdrawn to two **Background** tubes.

6. If **Background** samples are repeatedly used, mix reagents (**10 µl of PCR-mix-1-FL *HBV***, **5 µl of PCR-mix-2-FRT** and **0.5 µl of Polymerase TaqF** per one reaction) in a new sterile tube (see also Appendix 1, Part B). Thoroughly vortex the mixture. Make sure that there are no drops on the caps of the tubes.
7. Transfer **15 µl** of the prepared reaction mixture to each PCR tube. Add above **1** drop of **mineral oil for PCR.**
8. Add **10 µl** of **DNA samples** obtained from the clinical samples.

Avoid transferring sorbent beads together with the DNA sample in case of extraction by "RIBO-sorb", "MAGNO-sorb" kits, or "NucliSENS® easyMAG™" automated system.

9. Run the **control reactions**:

PCE - Add 10 **µl** of the **DNA sample** extracted from Positive Control-1-*HBV* to the tube labeled PCE (Positive Control of Extraction)

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

C+ - Add **10 µl** of **DNA calibrator PIC2 *HBV*** to the tube labeled C+ (Positive Control

of Amplification).

To rule out possible contamination, run an additional control reaction:

NCA -Add **10 µl** of **Buffer for elution** to the tube labeled NCA (Negative Control of Amplification).

Make sure that there are no drops on the tube walls, otherwise vortex tubes briefly.

8.2.2 Amplification.

Run the following program on the thermal cycler (see Table 1). When the temperature reaches 95 °C (pause mode), place tubes into cells of the thermal cycler, and start the amplification program.

It is recommended that drops are removed from walls of the tubes by short vortexing (1–3 s) before placing in the thermal cycler.

Table 1

HBV DNA amplification program

Thermal cyclers with active temperature adjustment*				Thermal cyclers with block temperature adjustment**			
Step	Temperature, °C	Time	Cycles	Step	Temperature, °C	Time	Cycles
1	**50**	30 min	1	1	**50**	30 min	1
2	**95**	15 min	1	2	**95**	15 min	1
3	**95**	2 s	45	3	**95**	10 s	45
	60	10 s			**60**	15 s	
					72	15 s	
4	**10**	storage		4	**10**	storage	

* For example, Terzik (DNA-Technology), Gradient Palm Cycler (Corbett Research), MaxyGene (Axygen), GeneAmp System 2400 (Perkin Elmer).

** For example, GeneAmp PCR System 2700 (Applied Biosystems), Palm-Cycler (Corbett Research), MaxyGene (Axygen).

Step 1 (50°C, 30 min) is required only when simultaneous amplification together with tests for *HCV* RNA, *HDV* RNA, *HIV* RNA, and *HCV* genotyping is performed.

9. DATA ANALYSIS

Detection is performed with ALA-1/4 florescence detector according to the protocol provided by the manufacturer (Biosan, Latvia).

The fluorescent signal intensity is detected in two channels:

– Signal from the Internal Control cDNA amplification product is detected in the FAM channel (or analogous, depending on the PCR instrument model);

– Signal from the *HBV* cDNA amplification product is detected in the HEX channel (or analogous, depending on the PCR instrument model).

Prior to detection, all settings should be entered and saved. Refer to the Guidelines and the Important Product Information Bulletin for settings.

Please read the ALA-1/4 Instrument Operating Manual before using this kit.

Program the detector according to manufacturer's manual and the Guidelines.

Results interpretation

1. When the analysis is complete, the results are automatically displayed in the table. Designations:

   pos – positive result;

   neg – negative result;

   eq – equivocal result (signal is in the grey zone);

   nd – invalid result (specific signal and IC signal are absent in the sample).

2. Principle of result interpretation:

   The value is considered **negativ**e if it is less than the defined threshold of negative result, **positive** if it is more than the defined threshold of positive result, and **equivocal** if it fits between the thresholds.

   – Positive result in the JOE/HEX channel indicates the presence of ***HBV*** **DNA** in the sample.

   – Result is invalid if the signal in the FAM channel is less than the defined threshold of negative result and the signal in the JOE/HEX channel is less than the defined threshold.

   – If the result is equivocal, it is necessary to perform PCR analysis of the sample once again.

3. The result of the analysis is considered reliable only if both Positive and Negative Controls are passed properly (Table 2).

Table 2

Results for controls

Control	Stage for control	Result of automatic interpretation		Interpretation
		FAM channel (IC)	JOE/HEX channel *(HBV)*	
C–	DNA isolation	**IC+**	neg	OK
PCE	DNA isolation	**IC+**	pos	OK
C+	Amplification	**IC+**	pos	OK
NCA	Amplification	**IC–**	nd	OK

10. TROUBLESHOOTING

If analysis results are not obtained as per the following examples:

- If the signal less than the threshold of positive result is detected for PCE or C+ in the JOE/HEX channel, then the analysis (starting from the isolation) should be repeated for all samples in which *HBV* DNA has not been found.
- If the signal more than the threshold of the positive result is detected for C– and/or NCA in the HEX channel, then the analysis (starting from the isolation) should be repeated for all samples in which *HBV* DNA has been found.
- If the **nd** result is obtained for samples except for NCA, the analysis should be repeated (starting from the isolation). The **nd** result is normal only for the NCA sample.
- If the **eq** result is registered for samples, the analysis should be repeated (starting from the isolation). If the same result obtained once again, the sample is considered positive.
- If positive signal is detected in C– and/or NCA, results of the analysis for all samples are considered invalid due to contamination. It is necessary to repeat the analysis of all tests and to take measures to detect and eliminate the source of contamination.

11. TRANSPORTATION

AmpliSens® *HBV*-FEP 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*-FEP** PCR kit are to be stored at temperature from minus 24 to minus 16 °C when not in use. All components of the **AmpliSens® *HBV*-FEP** 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.

DNA calibrator PIC2 *HBV*, Positive Control-*1-HBV* and the Internal Control IC STI-87 can be frozen/thawed at most twice. After thawing, these controls should be stored at 2–8 °C for 6 months.

PCR-mix-1-FL *HBV* is to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

The analytical sensitivity of **AmpliSens® *HBV*-FEP** PCR kit is specified in the table below.

Volume of sample for isolation, µl	DNA isolation method	Analytical sensitivity, IU/ml
100	RIBO-sorb RIBO-prep NucliSENS easyMAG	200
200	MAGNO-sorb	100
1000	MAGNO-sorb NucliSENS easyMAG	20

The claimed analytical features of **AmpliSens® *HBV*-FEP** PCR kit are guaranteed only when additional reagents kits, MAGNO-sorb, RIBO-sorb, or RIBO-prep (manufactured by Federal Budget Institute of Science "Central Research Institute for Epidemiology") are used.

13.2. Specificity.

The analytical specificity of **AmpliSens® *HBV*-FEP** PCR kit is ensured by selection of specific primers and probes 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 with genomic DNA/RNA of the following organisms and viruses: hepatitis A virus; hepatitis C virus; hepatitis D virus; human immunodeficiency virus; cytomegalovirus; Epstein-Barr virus; herpes simplex virus types 1 and 2; chicken pox virus; human herpes virus types 6 and 8; parvovirus B19; tick-borne encephalitis virus; West Nile encephalitis; adenovirus types 2, 3, and 7; *Escherichia coli*; *Staphylococcus aureus; Streptococcus pyogenes; Streptococcus agalactiae;* and *Homo sapiens*. The clinical specificity of **AmpliSens® *HBV*-FEP** PCR kit was confirmed in laboratory clinical trials.

14. REFERENCES

1. Handbook "Sampling, Transportation, 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, 2008.

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 **AmpliSens® *HBV*-FEP** 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
FBIS CRIE	Federal Budget Institute of Science “Central Research Institute for Epidemiology”	**C+**	Positive control of amplification
		IC	Internal control

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
01.06.10	7.1. DNA Isolation	Conditions of storage of purified DNA are added
	Page footer	Reference number is changed from V5-FEP to V5-FEP-CE
	4. Content, text	Name of Positive Control of amplification is changed from KB2 to PIC2
13.12.10	Through the text	Name of Positive Control of amplification is changed from Positive Control PIC2 *HBV* (C+) to DNA calibrator PIC2 *HBV*
03.07.11 RT	Cover page	The phrase “For Professional Use Only” was added
	Content	New sections “Working Conditions” and “Transportation” were added
		The “Explanation of Symbols” section was renamed to “Key to Symbols Used”
	Stability and Storage	The information about the shelf life of open reagents was added
		Information that PCR-mix-1-FL is to be stored away from light was added
	Key to Symbols Used	The explanation of symbols was corrected
	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”
15.09.11 RT	8. PROTOCOL 8.1. DNA isolation	The information about using RIBO-prep kit was added
13.06.12 LA	Cover page	Symbol IVD was replaced by RUO symbol
	16. Key to symbols used	
19.06.12 LA	13.1. Sensitivity	In the table of analytical sensitivity the unit of measurement was added: IU/ml
	8.1. DNA Isolation	Reference number of MAGNO-sorb reagent kit was changed from K2-16-1000 to K2-16-1000-CE
		Information about extraction with MAGNO-sorb is added