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For Professional Use Only

AmpliSens® *HBV*-FRT

PCR kit

Instruction Manual

AmpliSens®

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1. INTENDED USE

AmpliSens® *HBV*-FRT 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 using real-time hybridization-fluorescence detection.

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 the internal control sample (IC). The latter must be used in the isolation procedure to control the isolation of each individual sample and to detect possible reaction inhibition. *HBV* DNA detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using specific *HBV* 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. The real-time monitoring of fluorescence intensities during the real-time PCR allows detection of the amplified product without re-opening the reaction tubes after the PCR run. **AmpliSens® *HBV*-FRT** PCR kit uses “hot-start”, which greatly reduces the frequency of nonspecifically primed reactions. The “hot-start” is guaranteed by separation of nucleotides and Taq-polymerase by using a chemically modified polymerase (TaqF). The latter is activated by heating at 95 ºC 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*-FRT PCR kit is produced in one form:

AmpliSens® *HBV*-FRT PCR kit variant FRT (for use with RG, iQ, Mx, and Dt)

REF R-V5-Mod(RG,iQ,Mx,Dt)-CE.

AmpliSens® *HBV*-FRT 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
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*-FRT 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.
- Rotor-Gene 3000 or Rotor-Gene 6000 instrument (Corbett Research, Australia); or iCycler iQ5 instrument (Bio-Rad, USA); or Mx3000P instrument (Stratagene, USA).
- Disposable 0.2-ml polypropylene microtubes for PCR (for example, Axygen, USA).
- Refrigerator for 2–8 °C.
- Deep-freezer for ≤ –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 positive extracted 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, or 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 unidirectional; 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*-FRT** PCR kit is intended for analysis of 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 ≤ 68 °C) for a long time.

7. WORKING CONDITIONS

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

8. PROTOCOL

8.1. DNA Isolation

It is 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
- NucliSENS easyMAG automated system (BioMerieux) can also be used.

Isolate RNA/DNA according to the manual provided by the manufacturer.

To prepare 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 K2-1-Et-100-CE
- **0.28 ml** in the case of “MAGNO-sorb”, REF and 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
- Add 30 ml (the whole content of the bottle) of the **RT-G component from the EM-Plus kit** to the bottle with the NucliSens lysis buffer, close tightly the cap and **carefully** mix by turning upside down 7-10 times (this procedure is performed once for each reagent kit).
- Mix 10 µl of the **Internal Control (IC) sample with** 10 µl of **NucliSens magnetic silica** and 10 µl of **Component A** from the **EM-plus kit** with per one sample for RNA/DNA isolation in a new sterile tube using disposable tips with aerosol barriers.
- 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 mixture 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-ml amplification tubes for clinical and control samples (two controls of extraction and one control of amplification. The type of tubes depends on the real-time PCR instrument used for analysis.
3. **To prepare the reaction mixture**, mix the 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). Thoroughly vortex the mixture, make sure that there are no drops on the caps of the tubes.
4. Add **15 µl** of the prepared reaction mixture to each PCR tube.

5. Add **10 µl** of **DNA samples** isolated from the clinical samples to each PCR tube.

Avoid transferring sorbent beads together with the DNA sample in case of extraction using “RIBO-sorb” and “MAGNO-sorb” kits or the “NucliSENS easyMAG” automated system.

6. Run the **control reactions**:

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

C- - Add **10 µl** of the **DNA sample** extracted from the 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

8.2.2.1. RG

1. Program the Rotor-Gene instrument according to manufacturer’s manual and the Guidelines.
2. Create a temperature profile on your Rotor-Gene instrument as follows:

AmpliSens-2 RG program for rotor-type instruments[1]

Step	***Temperature*, °C**	***Time***	***Fluorescence detection***	**Cycles**
1 (Hold)	50	15 min	–	1
2 (Hold)	95	15 min	–	1
3 (Cycling 1)	95	5 s	–	5
	60	20 s	–	
	72	15 s	–	
4 (Cycling 2)	95	5 s	–	40
	60	20 s	FAM/Green, JOE/Yellow, ROX/Orange, Cy5/Red	
	72	15 s	–	

3. Adjust the fluorescence channel sensitivity as described in the Guidelines.

This program makes it possible to simultaneously carry out any combination of tests in

[1] For example, Rotor-Gene 3000 or 6000 (Corbett Research, Australia).

the same instrument using the single amplification program.
Step 1 (50 °C, 15 min) is required only when simultaneous amplification together with tests for *HCV* RNA, *HDV* RNA, and *HCV* genotyping is performed; otherwise, it can be omitted.

Channels ROX/Orange and Cy5/Red are switched on when necessary (only in MULTIPRIME assays)

8.2.2.2. iQ

1. Program the iCycler iQ™ or iQ™5 instrument according to manufacturer's manual and the Guidelines.
2. Create a temperature profile on your iQ5 instrument as follows:

AmpliSens-2 iQ program for plate-type instruments[2]

Step	***Temperature*, °C**	**Step duration**	***Fluorescence detection***	***Cycle repeats***
1	50	15 min	–	1
2	95	15 min	–	1
3	95	5 s	–	5
	60	20 c	–	
	72	15 s	–	
4	95	5 s	–	40
	60	30 s	FAM, HEX, ROX, Cy5	
	72	15 s	–	

3. Adjust the fluorescence channel sensitivity as described in the Guidelines.

This program makes it possible to simultaneously carry out any combination of tests in the same instrument using the single amplification program.
Step 1 (50 °C, 15 min) is required only when simultaneous amplification together with tests for *HCV* RNA, *HDV* RNA, and *HCV* genotyping is performed; otherwise, it can be omitted.

Channels ROX/Orange and Cy5/Red are switched on when necessary (only in MULTIPRIME assays)

9. DATA ANALYSIS

The signal from the Internal Control cDNA amplification product is detected in the FAM channel, the signal from the *HBV* cDNA amplification product is detected in the JOE/HEX channel.

For data analysis settings for Rotor-Gene 3000/6000, iCycler iQ5, Mx3000, and DT-96 real-time PCR instruments, see the Guidelines.

Results interpretation

The results are interpreted by the real-time PCR instrument software by the crossing or not crossing of the threshold line by the fluorescence curve.

Results for controls

[2] For example, iCycler iQ5 (Bio-Rad, USA), Mx3000P (Stratagene, USA), DT-96 (DNA-Technology, Russia), or equivalent.

Control	Stage for control	Ct in channel		Interpretation
		FAM	HEX/JOE	
C–	RNA isolation	Pos (≤ Ct*)	Neg	OK
PCE	RNA isolation	Pos (≤ Ct*)	Pos (≤ Ct*)	OK
NCA	Amplification	Neg	Neg	OK
C+	Amplification	Pos (≤ Ct*)	Pos (≤ Ct*)	OK

* The boundary Ct values are summarized in the Important Product Information Bulletin.

1. The sample is considered **positive** for *HBV* DNA if the Ct value detected in the JOE/HEX/Yellow channel does not exceed the boundary value specified in the Important product information bulletin.
2. The sample is considered **negative** for *HBV* DNA if the Ct value in the JOE/HEX/Yellow channel is absent or if the Ct value detected in the JOE/HEX/Yellow is greater than the specified boundary value and the Ct value in the FAM channel does not exceed the boundary value specified in the Important product information bulletin.
3. The sample is considered **equivocal** if an equivocal result is obtained in any of the channels. In this case, PCR analysis of this sample should be repeated once again.

For details, see the Guidelines.

Results are accepted as relevant if both positive and negative controls of amplification as well as negative and positive controls of extraction are passed properly (see the above table for controls).

10. TROUBLESHOOTING

- If the Ct value for PCE or C+ in the JOE/HEX/Yellow channel exceeds the specified boundary value, analysis of all samples in which *HBV* DNA was not detected should be repeated once again starting from the DNA extraction stage.
- If a Ct value for NCE and/or C– in the JOE/HEX/Yellow channel is detected and if this value does not exceed the specified boundary value, analysis of all samples in which *HBV* DNA was detected should be repeated once again starting from the DNA extraction stage.

11. TRANSPORTATION

AmpliSens® *HBV*-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*-FRT** 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*-FRT** 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 2–8ºC for at most 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*-FRT** PCR kit is specified in the table below.

Volume of sample for isolation, µl	DNA isolation method	Analytical sensitivity, IU/ml
100	RIBO-sorb	100
	RIBO-prep NucliSENS easyMAG	50
200	MAGNO-sorb	50
1000	MAGNO-sorb	10
	NucliSENS easyMAG	5

The claimed analytical features of **AmpliSens® *HBV*-FRT** 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*-FRT** PCR kit is ensured by selection of specific primers and probes and strict reaction conditions. The primers and probes were tested 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*-FRT** PCR kit was confirmed in laboratory clinical trials.

Cross-reactions for the above-mentioned organisms and viruses have not been detected.

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.

2. Guidelines to AmpliSens® *HCV*-FRT, AmpliSens® *HDV*-FRT, and AmpliSens® *HBV*-FRT PCR kits.

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*-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
FBIS CRIE	Federal Budget Institute of Science “Central Research Institute for Epidemiology”	**C+**	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
31.05.10	7.1. DNA Isolation	Conditions of storage of purified DNA are changed
	Page footer	Reference number is changed from R-V5-Mod(RG,iQ,Mx,Dt)-E to R-V5-Mod(RG,iQ,Mx,Dt)-CE
	3. Content, text	Name of Positive Control of amplification is changed from KB2 to PIC2
03.08.10	3. Content	The number of Positive Control PIC2 *HBV* (C+) tubes is changed from 1 to 4
10.12.10	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”
	13. Specifications 13.1. Sensitivity	The table of analytical sensitivity was corrected in accordance with Russian instruction
15.09.11 RT	8. PROTOCOL 8.1. DNA extraction	The information about using RIBO-prep kit was added
26.09.11 LA	8.2.2. Amplification	Notes below amplification program tables were corrected
13.06.12 LA	Cover page	Symbol IVD was replaced by RUO symbol
	16. Key to symbols used	
19.06.12 LA	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