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For Professional Use Only

AmpliSens® *HBV*-EPh

PCR kit

Instruction Manual

AmpliSens®

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TABLE OF CONTENTS

1. INTENDED USE

AmpliSens® *HBV*-EPh PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *hepatitis B virus (HBV)* DNA in the clinical material (blood plasma) using electrophoretic detection of the amplified products in agarose gel.

2. PRINCIPLE OF PCR DETECTION

Hepatitis B virus detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen DNA specific region using specific *HBV* primers. After PCR the amplified product is detected in agarose gel. **AmpliSens® *HBV*-EPh** PCR kit is a qualitative test and contains the IC that must be used in the extraction procedure in order to control the extraction process of each individual specimen and to identify possible reaction inhibition.

AmpliSens® *HBV*-EPh PCR kit uses "hot-start" which greatly reduces frequency of nonspecifically primered reactions. Hot-start is guaranteed by separation of nucleotides and Taq-polymerase by wax layer. Wax melts and reaction components mix only at 95ºC.

3. CONTENT

AmpliSens® *HBV*-EPh PCR kit is produced in 2 forms:

AmpliSens® *HBV*-EPh PCR kit variant 100 R (0.5-ml tubes), **REF** V5-100-R0,5-CE.

AmpliSens® *HBV*-EPh PCR kit variant 100 R (0.2-ml tubes), **REF** V5-100-R0,2-CE.

AmpliSens® *HBV*-EPh PCR kit variant 100 R includes:

Reagent	***Description***	***Volume, ml***	***Amount***
PCR-mix-1-R *HBV* ready-to-use single-dose test tubes (*under wax)*	colorless clear liquid	0.005	110 tube of 0.5 or 0.2 ml
PCR-mix-2 blue	blue clear liquid	1.2	1 tube
Mineral oil for PCR	colorless viscous liquid	4.0	1 vial
Positive Control DNA *HBV* (C+ $_{HBV}$)*	colorless clear liquid	0.2	1 tube
DNA-buffer	colorless clear liquid	0.5	1 tube
Negative Control (C-)**	colorless clear liquid	1.2	1 tube
Internal Control *HBV* (IC)***	colorless clear liquid	1.0	1 tube

* must be used in the extraction procedure as Positive Control of Extraction;

** must be used in the extraction procedure as Negative Control of Extraction;

*** add 10 µl of Internal Control during the extraction procedure directly to the sample/lysis mixture (see "RIBO-sorb" **REF** K2-1-Et-100-CE or "RIBO-prep" **REF** K2-9-Et-100-CE

protocols).

AmpliSens® *HBV*-EPh PCR kit variant 100 R is intended for 110 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- RNA/DNA extraction kit.
- Agarose gel detection kit.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol barriers (up to 200 µl).
- Vortex mixer.
- PCR box.
- Thermostatic bath or dry block for tubes with controlled temperature and capability to incubate at temperature between 25 °C and 100 °C.
- Tube racks.
- Personal thermocyclers (for example, “Gradient Palm Cycler” (Corbett Research, Australia), “GeneAmp PCR System 2700” (Applied Biosystems), GeneAmp PCR System 2400” (Applied Biosystems), “Maxygene” (Axygen), “Terzik” (DNA-Technology), “Uno-2” (Biometra), “MiniCycler”, “PTC-100”(MJ Research), “Maxygene” (Axygen).
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity (for example, “Axygen”, USA).
- Refrigerator with temperature between 2 and 8 °C.
- Deep-freezer with temperature below minus16 °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 and handle amplicons away from all other reagents.
- Thaw all components thoroughly at room temperature before starting detection.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats, protect eyes while samples and reagents handling. Thoroughly wash hands afterward.
- 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 samples and unused reagents in compliance with local authorities requirements.
- Samples should be considered potentially infectious and handled in a biological cabinet in accordance 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 mucosa. If skin, eyes and mucosa contact immediately flush with water, 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

Sampling biological materials for PCR-analysis, transportation, and storage are described in detail in handbook of the manufacture [1]. It is recommended that this handbook is read before beginning work.

AmpliSens® *HBV*-EPh PCR kit is intended to analyze DNA extracted with RNA/DNA extraction kits from:

- *Blood plasma*

Peripheral blood should be collected in a tube that contains 6% EDTA solution (50 µl of EDTA per 1.0 ml of blood) after overnight fasting. When the tube is filled, invert it several times to ensure adequate mixing. Spin the tube at 3,000 r/min for 10 min. Remove and transfer plasma in a 1.5-ml tube. Plasma should be collected within 6 h from the time of blood taking.

Storage of plasma samples:

- at 2– 8 °C for up to 1 week;
- at or below –68°C for 1 year.

Only one freeze-thaw cycle of clinical material is allowed.

7. WORKING CONDITIONS

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

8. PROTOCOL

8.1. RNA/DNA extraction

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.

Please carry out the RNA/DNA extraction according to the manufacturer's protocol.

Transfer 10 µl of Internal Control *HBV* per each extraction tube just before lysis solution adding.

Positive Control DNA *HBV* must be used during extraction procedure.
Add 10 µl of Positive Control DNA *HBV* and 90 µl of Negative Control (C-) in the tube for Positive Control of Extraction.

8.2. Preparing the PCR

Total reaction volume – 25 µl, volume of a DNA sample – 10 µl.

8.2.1 Preparing tubes for PCR

1. Prepare the required number of tubes with **PCR-mix-1-R *HBV*** with wax for amplification of DNA of clinical and control samples.
2. Add **10 µl of PCR-mix-2 blue** to the surface of wax layer, so that it does not fall under the wax and mix with PCR-mix-1-R *HBV*.
3. Add above 1 drop of **mineral oil for PCR** (about 25 µl).

8.2.2 Amplification

Use prepared tubes for PCR. Under or immediately above the oil **add 10 µl of DNA sample** obtained from clinical or control samples at the stage of DNA extraction. Use tips with aerosol barriers.

Carry out **control amplification reactions:**

NCA -Add 10 µl of **DNA-buffer** to the tube for Negative Control of Amplification (NCA).

C+ -Add 10 µl of **Positive Control DNA *HBV*** to the tube for Positive Control of Amplification (C+).

Run the following program on the thermocycler (see table 1). When the temperature will reach 95ºC (pause regimen), insert tubes into cells of the thermocycler and press the button to continue.

It is recommended to sediment drops from tube walls by short vortexing (1–3 s) before inserting them in the thermocycler.

Table 1

Amplification program for *HBV* DNA

	Thermocyclers with active temperature adjustment:								
	GeneAmp PCR System 2400 (Applied Biosystems); Terzik (DNA-Technology)			**GeneAmp PCR System 2700 (Applied Biosystems); Gradient Palm Cycler (Corbett Research)**			**Maxygene (Axygen)**		
step	tempera ture	time	cycles	tempera ture	time	cycles	tempera ture	time	cycles
0	**95 °C**	pause		**95 °C**	pause		**95 °C**	pause	
1	**95 °C**	2 min	1	**95 °C**	2 min	1	**95 °C**	5 min	1
2	**95 °C**	10 s	42	**95 °C**	20 s	42	**95 °C**	30 s	42
	65 °C	10 s		**65 °C**	20 s		**65 °C**	45 s	
	72 °C	10 s		**72 °C**	20 s		**72 °C**	45 s	
3	**72 °C**	1 min	1	**72 °C**	1 min	1	**72 °C**	1 min	1
4	**4 °C**	storage		**4 °C**	storage		**10 °C**	storage	

Amplification program for *HBV* cDNA

	Thermocyclers with block temperature adjustment: **MiniCycler, PTC-100 (MJ Research)**		
step	temperature	time	cycles
0	**95 °C**	pause	
1	**95 °C**	2 min	1
2	**95 °C**	1 min	42
	67 °C	1 min	
	72 °C	1 min	
3	**72 °C**	1 min	1
4	**4 °C**	storage	

The run takes approximately 2 h 30 min to complete in a thermocycler with block temperature adjustment or 1 h 50 min in a thermocycler with active temperature adjustment.

After the reaction is completed, the PCR tubes must be collected and sent to the room for PCR products analysis.

Analysis of amplification products is performed by separation of DNA fragments in agarose gel.

The amplified samples can be stored at room temperature for 16 h or at 2 – 8 °C for 1 week (make sure that samples are warmed up to room temperature before running electrophoresis).

9. DATA ANALYSIS

It's recommended that the following detection agarose kit is used:

- "EPh" variant 200, **REF** K5-200-CE.

Analysis results are based on the presence or absence of specific bands of amplified DNA in the agarose gel (1.7%). The length of specific amplified DNA fragments is:

- ***HBV* – 470 bp**
- **IC *HBV* – 770 bp**

Use a protective mask or a glass filter when looking at the gel or taking photos

9.1. Interpretation of results

Table 2

Results for controls

Control	Step for control	Specific bands in the agarose gel		Interpretation
		470 bp	770 bp	
PCE	DNA extraction	**Yes**	**Yes**	OK
C-	DNA extraction	No	**Yes**	OK
NCA	Amplification	No	No	OK
C+	Amplification	**Yes**	No	OK

- The sample is considered positive for *hepatitis B virus* DNA if the 470-bp band is present in agarose gel. The band of IC (770 bp) may be absent in the samples with high concentration of *HBV* DNA.
- The sample is considered negative for *hepatitis B virus* DNA if the 470-bp band is absent and the 770-bp band is present in the gel.

Besides the specific products, the fuzzy bands of primer dimers may appear in the lanes below the 100-bp level.

10. TROUBLESHOOTING

Analysis results are not taken into account in the following cases:

- If the results of the controls do not match with the listed above (Table 2), then the appropriate step of the test should be repeated.
- If none of the 470- and 770-bp bands is observed in the lane of the clinical sample, the result of analysis for this sample is invalid. It can be caused by errors in sample processing that led to the loss of DNA or inhibition of PCR. The test should be repeated from DNA extraction stage.
- If nonspecific bands are seen at different levels in the lanes the result of analysis is invalid. It may be caused by lack of “hot start” or incorrect temperature profile of the thermocycler. The PCR should be repeated.
- If the specific 470-bp band appears in the lane corresponding to a negative control (NCA, C–), it indicates contamination of the reagents or samples. In this case results of the analysis for all samples are considered invalid. The test must be repeated and measures to detect and eliminate the source of contamination should be taken.

11. TRANSPORTATION

AmpliSens® *HBV-EPh* PCR kit should be transported at 2–8 ºC for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the AmpliSens® *HBV*-EPh PCR kit should be stored at 2 ºC to 8 ºC when not in use. All components of the AmpliSens® *HBV*-EPh PCR kit are stable until the labeled expiration date. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

13. SPECIFICATIONS

13.1. Sensitivity

Analytical Sensitivity of AmpliSens® *HBV*-EPh PCR kit is no less than 1 x 10^3 copies per 1 ml of plasma (copies/ml).

Claimed analytical performance characteristics of AmpliSens® *HBV*-EPh PCR kit are guaranteed only when additional reagent kits, “RIBO-sorb” or “RIBO-prep”, and “EPh”, are used.

13.2. Specificity

The analytical specificity of AmpliSens® *HBV*-EPh PCR kit is ensured by selection of specific primers and strict reaction conditions.

The clinical specificity of AmpliSens® *HBV*-EPh 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”, 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*-EPh PCR kit is 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	**PCE**	Positive Control of Extraction
	Manufacturer	**NCA**	Negative control of amplification
	Date of manufacture	**C–**	Negative control of extraction
IC	Internal control	**C+**	Positive control of amplification

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
29.06.11 VV	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”
	Cover page	The phrase “For Professional Use Only” was added
	Content	The “Explanation of Symbols” section was renamed to “Key to Symbols Used”
	Key to Symbols Used	The explanation of symbols was corrected
12.07.11 VV	Content	New sections “Working conditions “and “Transportation” were added
	Stability and Storage	The information about the shelf life of open reagents was added
16.04.12. Ivl	Title page, Key to symbols used	Symbol IVD *in vitro* diagnostic medical device was changed to RUO research use only