



For *in Vitro* Diagnostic Use

TABLE OF CONTENTS

1. INTENDED USE3

2. PRINCIPLE OF PCR DETECTION3

3. CONTENT3

4. ADDITIONAL REQUIREMENTS4

5. GENERAL PRECAUTIONS4

6. SAMPLING AND HANDLING5

7. PROTOCOL5

8. DATA ANALYSIS8

9. TROUBLESHOOTING9

10. STABILITY AND STORAGE 10

11. SPECIFICATIONS 10

12. REFERENCES 11

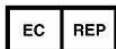
13. QUALITY CONTROL 11

14. EXPLANATION OF SYMBOLS 11

AmpliSens® HBV-Monitor-FRT

PCR kit

Instruction Manual



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1. INTENDED USE

AmpliSens® *HBV*-Monitor-FRT PCR kit is an *in vitro* nucleic acid amplification test for quantitative detection of *HBV* DNA in clinical material (blood plasma) by means of real-time hybridization-fluorescence detection.

2. PRINCIPLE OF PCR DETECTION

HBV detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region by using specific 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 PCR monitoring of the fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening of the reaction tubes after the PCR run.

AmpliSens® *HBV*-Monitor-FRT PCR kit uses “hot-start”, which greatly reduces 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 95°C for 15 min.

3. CONTENT

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

Form 1 includes RIBO-prep nucleic acid extraction kit and AmpliSens® *HBV*-Monitor-FRT PCR kit variant FRT, **REF** TR-V5-P-M(RG,iQ,Mx,Dt)-CE

Form 2 includes AmpliSens® *HBV*-Monitor-FRT PCR kit variant FRT and *HBV*-Q calibration kit, **REF** R-V5-MC(RG,iQ,Mx,Dt)-CE

AmpliSens® *HBV*-Monitor-FRT PCR kit variant FRT includes:

Reagent		Description	Volume (ml)	Quantity
PCR-mix-1-FL <i>HBV</i>		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-calibrators	PIC1 <i>HBV</i> (C ₁)	colorless clear liquid	0.1	4 tubes
	PIC2 <i>HBV</i> (C ₂)	colorless clear liquid	0.1	4 tubes
Buffer for elution		colorless clear liquid	1.2	4 tubes

Negative Control (C-)*	colorless clear liquid	1.2	4 tubes
Positive Control-1- <i>HBV</i> **	colorless, clear liquid	0.06	4 tubes
Positive Control-2- <i>HBV</i> **	colorless, clear liquid	0.06	4 tubes
Internal Control STI-87 (IC)***	colorless, clear liquid	0.28	4 tubes

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

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

*** must be added during the DNA extraction procedure.

AmpliSens® *HBV*-Monitor-FRT PCR kit is intended for 80 reactions, including controls and calibrators.

HBV-Q calibration kit includes:

Reagent	Description	Volume (ml)	Quantity
Calibrator <i>HBV</i> -Q	yellow powder	–	1 tube
Solvent Q	colorless, clear liquid	1.2	3 tubes

4. ADDITIONAL REQUIREMENTS

- DNA/RNA extraction kit.
- Disposable powder-free gloves.
- 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.
- Rotor-Gene™ 3000 or Rotor-Gene™ 6000 (Corbett Research, Australia), iQ5 (BioRad, USA), or Mx3000 (Stratagene, USA) 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 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 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 move to the Amplification and Detection Area. 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 biological material samples for PCR-analysis, transportation, and storage are described in manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

AmpliSens® HBV-Monitor-FRT PCR kit is intended for the analysis of DNA extracted using DNA/RNA extraction kits from:

- *Peripheral blood plasma*

Collect blood samples into tubes with 3% EDTA solution (1 : 20) after overnight fasting. Invert closed tubes to ensure proper mixing. To collect plasma, centrifuge the tubes with blood at 800–1600 g for 20 min within 6 h after blood taking. Remove obtained plasma and transfer to new tubes.

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.

Storage of plasma and serum samples:

- from 2 to 8°C for up to 3 days
- at or below 68°C for a long time.

7. PROTOCOL

7.1. DNA extraction

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

- “RIBO-prep”, **REF** K2-9-Et-100-CE

- “MAGNO-sorb”, **REF** K2-16-1000-CE

- “NucliSENS® easyMAG™” automatic device can be used either.

Carry out the DNA extraction according to the manual provided by the manufacturer.

Volume of Internal Control *ICZ-rec* added during DNA extraction is **10 µl** per one sample.



If “NucliSENS® easyMAG™” automated system is applied:

- use of “EM-plus” kit **REF** K2-15-96-CE (manufactured by CRIE) is obligatory
- set a sample volume as 0.1 ml or 1 ml;
- set an eluate volume as 55 µl.
- both *On-board* and *Off-board* Lysis Buffer Dispensing and Lysis Incubation are possible.

See Guidelines [2] for details.

7.2. Preparing the PCR

The total reaction volume is **50 µl**, the volume of DNA sample is **25 µl**.

7.2.1 Preparing tubes for PCR



All components of the reaction mixture should be mixed just before use. See Appendix 1 for the reaction mixture preparation scheme.

1. Thaw all reagents, thoroughly vortex, and make sure that there are no drops on the walls of the tubes.
2. Collect the required number of the PCR tubes for amplification of clinical and control samples (including 3 controls of extraction and 4 calibrators).
3. To prepare the **reaction mixture**, take a new tube and mix the following reagents calculating per one reaction: **15 µl of PCR-mix-1-FL HBV**, **10 µl of PCR-mix-2-FRT**, and **1.0 µl of polymerase (TaqF)**. Vortex thoroughly and make sure that there are no drops on the walls of the tubes. It is recommended that the reaction mixture for 20 reactions is prepared in case of isolation from 12 to 16 samples (two “NucliSENS® easyMAG™” arrays). To do this, into the tube with **PCR-mix-1-FL HBV** transfer the entire contents of the tube with **PCR-mix-2-FRT and polymerase (TaqF)**. Do not store the prepared mixture!
4. Transfer 25 µl of the prepared mixture per each PCR tube. Discard unused reaction mixture.
5. Using tips with aerosol barrier add **25 µl** of clinical **DNA-samples**. Thoroughly mix by pipeting. Avoid air bubbling.



Avoid transferring of the sorbent together with the DNA sample in case of extraction with “MAGNO-sorb” kit or “NucliSENS® easyMAG™” automated system

6. Carry out the control amplification reactions:

- PCE 1** -Add **25 µl of DNA sample** extracted from Positive Control-1-*HBV* to the tube for positive control of extraction 1;
- PCE 2** -Add **25 µl of DNA sample** extracted from Positive Control-2-*HBV* to the tube for positive control of extraction 2;
- C-** -Add **25 µl of DNA sample** extracted from Negative Control to the tube for negative control of extraction;
- C+₁** -Add **PIC1 *HBV*** to two tubes for the positive control of amplification 1 (**25 µl per each tube**);
- C+₂** -Add **PIC2 *HBV*** to two tubes for the positive control of amplification 2 (**25 µl per each tube**).

Thoroughly mix by pipeting. Avoid air bubbling.

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

NCA -Add **25 µl of Buffer for elution** to the tube for negative control of amplification.

7.2. 2 Amplification

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

AmpliSens-2 RG program (for rotor-type instruments)

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

AmpliSens-2 iQ program (for plate-type instruments)

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



It is possible to carry out any combination of tests that apply AmpliSens-2 RG or AmpliSens-2 iQ program (for example, *HDV* test, *HCV*-genotyping, etc.) within the same run.

If only *HBV* DNA is analyzed, the first step (50 °C – 15 min) can be omitted.



ROX/Orange and Cy5/Red channels are activated as may be required for “multiprime” format tests.

4. See the Guidelines for the settings.

8. DATA ANALYSIS

Results interpretation

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

The results are interpreted by the crossing (or not crossing) of the fluorescence curve with the threshold line set at a certain level.

See the Guidelines for Rotor-Gene™ 3000/6000, iQ5, and Mx3000 data analysis settings.

Calibration line plotting as well as calculation of Positive Control and Internal Control concentrations are based on the obtained Ct values and specified calibrator’s values (PIC1 and PIC2). *HBV* DNA concentration is calculated according to the following formula:

$$\frac{\text{HBV DNA copies per PCR sample}}{\text{IC DNA copies per PCR sample}} \times \text{coefficient A} \times \text{coefficient B} = \text{IU HBV DNA/ml of plasma}$$

$$\text{Coefficient A} = \frac{100}{\text{extraction volume, } \mu\text{l}}$$



Coefficient A = 1 in calculating PCE 1 and PCE 2 concentrations

Coefficient B (IC copies/ml of plasma) is specified in the Important Product Information Bulletin enclosed in the PCR kit and specific for each lot.

If DNA is obtained with an extraction kit which is not included in this PCR kit, then coefficient B should be calculated as the result of calibration during the first PCR run (see Appendix 2 for details).

If the result is more than 100,000,000 IU/ml, then it will be displayed as the **result more than 100,000,000 IU *HBV*/ml**. If the result is more than the linear measurement range, the sample can be analyzed after 10x dilution and the obtained result should be multiplied by 10.

If the result is less than 150 IU/ml in case of extraction from 100 µl, less than 75 IU/ml in case of extraction from 200 µl, or less than 15 IU/ml in case of extraction from 1 ml, then it will be displayed as the **result less than 150, less than 75, or less than 15 IU HBV/ml**, respectively.

The result calculated in IU/ml can be converted into copies/ml by multiplying by 1.7 (1 IU = 1.70 copies/ml, 1 copy = 0.59 IU).



Ct boundary values are specified in the Important product information bulletin enclosed in the PCR kit.

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

Results for controls

Control	Stage for control	Ct in channel		Interpretation
		FAM	HEX/JOE	
C-	DNA extracton	Pos (Ct ≤ value specified in the Bulletin)	Neg	OK
PCE 1	DNA extraction	Pos (Ct ≤ value specified in the Bulletin)	Pos (should fall in the range specified in the Bulletin as a result of calculation with IC copies/ml)	OK
PCE 2	DNA extraction	Pos (Ct ≤ value specified in the Bulletin)	Pos (should fall in the range specified in the Bulletin as a result of calculation with IC copies/ml)	OK
C+ ₁	Amplification	Pos	Pos	OK
C+ ₂	Amplification	Pos	Pos	OK
NCA	Amplification	Neg	Neg	OK

9. TROUBLESHOOTING

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

- if the Ct value obtained for the positive controls of extraction or amplification (PCE or C+) in the JOE/HEX/Yellow channels is more than the specified boundary value. Repeat the test (from DNA extraction) for all samples in which HBV DNA is not found.
- if the Ct value obtained for the negative control of extraction (C-) and/or negative control of amplification (NCA) in the JOE/HEX/Yellow channel is less than the specified boundary value. Repeat the test (from DNA extraction) for all samples in which HBV DNA is found.
- if the correlation coefficient, R², is less than 0.98 when the calibration line is plotted. Repeat PCR for all samples.
- if the calculated concentrations of Positive Control-1-HBV and Positive Control-2-HBV do not fall in the range specified in the Important Product Information Bulletin. Repeat the test (from DNA extraction) for all samples.

10. STABILITY AND STORAGE

All components of the **AmpliSens® HBV-Monitor-FRT** PCR kit are to be stored at or below minus 16 °C when not in use. All components of the **AmpliSens® HBV-Monitor-FRT** PCR kit are stable until the expiration date stated on the label.



Do not repeat freeze-thaw cycles more than twice for Positive Control-1-HBV, Positive Control-2-HBV, PIC1 HBV, PIC2 HBV, Internal Control STI-87. Store the above-mentioned reagents at 2–8 °C for up to 6 month after thawing.

11. SPECIFICATIONS

11.1. Sensitivity

The linear measurement range of **AmpliSens® HBV-Monitor-FRT** PCR kit is specified in the table below.

Volume of sample for extraction, µl	DNA/RNA extraction kit	Linear measurement range, IU/ml
100	RIBO-sorb RIBO-prep NucliSENS® easyMAG™	150 – 100,000,000
200	MAGNO-sorb	75 – 100,000,000
1000	MAGNO-sorb NucliSENS® easyMAG™	15 – 100,000,000

11.2. Specificity

The analytical specificity of **AmpliSens® HBV-Monitor-FRT** PCR kit is ensured by selection of specific primers and probes as well as by selection of strict reaction conditions. The primers and probes were checked for possible homologies to all in sequences published gene banks by sequence comparison analysis as well as with genomic DNA/RNA of the following organisms and viruses: *hepatitis A virus*; *hepatitis D virus*; *hepatitis C 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*. No cross-reaction was observed for the aforementioned organisms and viruses.

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

















12. REFERENCES

1. Handbook "Sampling, transportation, storage of clinical material for PCR diagnostics", developed by Federal State Institution of Science Central Research Institute of Epidemiology of Federal Service for Surveillance on Consumers' Rights Protection and Human Well-Being, Moscow, 2008.
2. Guidelines to AmpliSens[®] HCV-Monitor-FRT and AmpliSens[®] HBV-Monitor-FRT PCR kits.

13. QUALITY CONTROL

In compliance with Federal State Institution of Science "Central Research Institute of Epidemiology" ISO 13485 – certified Quality Management System, each lot of **AmpliSens[®] HBV-Monitor-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

14. EXPLANATION OF SYMBOLS

	Manufacturer		Temperature limitation
	Use by		Batch code
	For <i>in Vitro</i> Diagnostic Use		Version
	Catalogue number		Internal Control complex
	Contains sufficient for <n> tests		Authorized representative in the European Community.
	Consult instructions for use		Caution, consult accompanying documents
	For working with Rotor-Gene™ 3000/6000		For working with IQ5, IQ.iCycler
	Positive control		Negative control