RUO

For Professional Use Only

AmpliSens® *HBV / HDV*-FRT

PCR kit

Instruction Manual

AmpliSens®

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

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1. INTENDED USE

AmpliSens® ***HBV / HDV*-FRT** PCR kit is an *in vitro* nucleic acid amplification test for simultaneous detection of *hepatitis B virus* (*HBV)* DNA and *hepatitis D virus* (*HDV)* RNA in 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 and *hepatitis D virus* (*HDV)* RNA are extracted from blood plasma together with internal control sample (IC). Detection by the polymerase chain reaction (PCR) is based on the reverse transcription of DNA/RNA and amplification of pathogen genome specific region using specific primers. In real-time PCR the amplified product is detected using fluorescent dyes. These dyes are usually linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. The real-time PCR monitoring of the 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 / HDV*-FRT** PCR kit is a qualitative test which contain the Internal Control (IC). It must be used in the extraction procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition. **AmpliSens® *HBV / HDV*-FRT** 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 95 °C for 15 min.

The IC amplification product is detected in the FAM channel. The *HBV* DNA amplification product is detected in the JOE channel. The *HDV* cDNA amplification product is detected in the ROX channel. The Positive Control of Extraction, Positive Control *HBV* / *HDV*-rec, is detected in FAM (IC), JOE (*HBV*) and ROX (*HDV*) channels. The Positive Control of Amplification, Positive Control cDNA *HBV* / *HDV*-FL, is the complex control for *HBV*, *HDV* and IC. It is detected in FAM (IC), JOE (*HBV*) and ROX (*HDV*) channels.

3. CONTENT

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

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

REF R-V56(RG,iQ,Mx,Dt)-CE.

AmpliSens® *HBV / HDV*-FRT PCR kit variant FRT in bulk[1] (for use with RG, iQ, Mx, Dt)

REF R-V56(RG,iQ,Mx,Dt)-CE-B.

AmpliSens® *HBV / HDV*-FRT PCR kit variant FRT includes:

Reagent	***Description***	***Volume (ml)***	***Quantity***
RT-G-mix-2	colorless clear liquid	0.015	4 tubes
RT-PCR-mix-1-FL *HBV / HDV*	colorless clear liquid	0.3	4 tubes
RT-PCR-mix-2-FEP/FRT	colorless clear liquid	0.2	4 tubes
Polymerase (TaqF)	colorless clear liquid	0.02	4 tubes
TM-Revertase (MMlv)	colorless clear liquid	0.01	4 tubes
Positive Control cDNA *HBV / HDV*-FL (C+*HBV / HDV*-FL)	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 *HBV / HDV*-rec**	colorless clear liquid	0.06	4 tubes
Internal Control *ICZ*-rec***	colorless clear liquid	0.28	4 tubes

* 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 / HDV*-FRT PCR kit is intended for 112 reactions, including controls.

4. ADDITIONAL REQUIREMENTS

- RNA/DNA extraction kit
- Disposable powder-free gloves and laboratory coat
- Automated pipettors (dosers) of variable volumes
- Sterile RNase/DNase-free pipette tips with filters (up to 200 µl)
- Tube racks
- Centrifuge/vortex mixer
- PCR box
- Personal thermocyclers (for example, Rotor-Gene 3000 or Rotor-Gene 6000 (Corbett Research, Australia); iQ5 (Bio-Rad, USA), Mx3000P (Stratagene, USA)) or equivalent)

[1] In bulk form contains unlabeled tubes. Tubes with identical reagent are packed in one bag with label.

- Disposable polypropylene microtubes for PCR with 0.2 or 0.1 ml capacity (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 filters 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 samples of biological materials 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 / HDV*-FRT PCR kit is intended for the reverse transcription of RNA and amplification of cDNA extracted by RNA/DNA extraction kits from peripheral blood plasma.

- *Peripheral blood plasma*.

Blood samples are taken after overnight fasting into the tube with EDTA solution as anticoagulant. 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 for such material is the same but the clinical sensitivity can be reduced in view of viral particles coprecipitation during 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 / HDV*-FRT PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. RNA Extraction

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

- RIBO-sorb, REF K2-1-Et-50-CE (2 kits)
- RIBO-prep, REF K2-9-Et-50-CE (2 kits)
- MAGNO-sorb, REF K2-16-1000-CE
- Automated system NucliSENS easyMAG can also be used.

Carry out the RNA/DNA extraction according to the manufacturer's instructions.

For Positive Control of extraction (**PCE**), mix **10 µl** of **Positive Control *HDV/HDV*-rec** and **90 µl Negative Control**

Volume of Internal Control added during RNA/DNA extraction depends on the reagents kit used:

- add 10 µl of Internal Control *ICZ*-rec to the sample/lysis mixture (RIBO-prep or RIBO-sorb)

If using RIBO-sorb kit, it is necessary to incubate tubes with sample/lysis mixture (before sorbent adding) at 60 °C for 10 min and then centrifuge briefly.

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 RNA from blood plasma sample of 1000 µl, the volume of the **Internal Control *ICZ*-rec** required for extraction from 24 samples is **0.28 ml**.
- 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 NucliSENS easyMAG automated system is applied:

- use of EM-plus kit REF K2-15-96 (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** per one sample for RNA/DNA extraction in a new sterile tube using disposable tips with aerosol barriers.
- set a sample volume as 0.1 ml or 1 ml;
- set an eluate volume as 50-60 µl (up to 100 µl).
- both On-board and Off-board Lysis Buffer Dispensing and Lysis Incubation are possible.

See Guidelines for details.

8.2. Preparing the PCR

Total reaction volume is **25 µl**, the volume of RNA/DNA sample is **10 µl**.

8.2.1 Preparing tubes for PCR

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.

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 tubes for amplification for the clinical and control samples (two controls of extraction and one control of amplification). The type of tubes depends on the PCR instrument used for analysis.
3. To prepare the reaction mixture, mix reagents **10 µl of RT-PCR-mix-1-FL *HBV* / *HDV*, 5 µl of RT-PCR-mix-2-FEP/FRT, 0.25 µl of RT-G-mix-2, 0.5 µl of polymerase (TaqF) and**

0.25 µl of TM-Revertase (MMlv) per one reaction in a new sterile tube. Thoroughly vortex the mixture, make sure that there are no drops on the caps of the tubes.

4. Transfer **15 µl** of prepared mixture into each tube.
5. Using tips with filters add **10 µl** of **RNA/DNA** obtained from clinical samples.

When adding of RNA/DNA samples extracted by RIBO-sorb and NucliSENS easyMAG avoid transferring the sorbent into the reaction mix.

6. Carry out the control amplification reactions:

PCE - Add **10 µl** of **RNA/DNA sample** extracted from **Positive Control *HBV* / *HDV*-rec** sample to the tube labeled NCA (Positive Control of Extraction).

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

C+*HBV / HDV*-FL - Add **10 µl** of **Positive Control cDNA *HBV* / *HDV*-FL** to the tube labeled C+*HBV / HDV*-FL (Positive Control of Amplification).

To rule out possible contamination, carry out additional control reaction:

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

8.2. 2. Amplification

8.2.2.1. RG

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

AmpliSens-1 RG amplification program

Step	***Temperature, °C***	***Time***	***Fluorescence detection***	***Cycle repeats***
1 (Hold)	50	15 min	–	1
2 (Hold)	95	15 min	–	1
3 (Cycling)	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	–	

AmpliSens-1 RG general program allows simultaneous conducting of tests for *HDV* detection with *HBV*, *HCV* typing or others

Cy5/Red channel is switched on If needs for "multiprime" format tests.

3. Make the adjustment of the fluorescence channel sensitivity according to guidelines.

8.2.2.2. iQ5

1. Program the iQ according to manufacturer's manual and guidelines.

2. Create a temperature profile on your iQ instrument as follows:

AmpliSens-1 iQ program

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	–	

AmpliSens-1 iQ general program allows simultaneous conducting of tests for *HDV* detection with *HBV*, *HCV* typing or others

3. Make the adjustment of the fluorescence channel sensitivity according to guidelines.

9. DATA ANALYSIS

Internal Control is detected in the FAM fluorescence channel, *HBV* DNA is detected in the JOE fluorescence channel, *HDV* cDNA is detected in the ROX fluorescence channel.

See guidelines for data analysis settings for used instrument.

Results interpretation

The results are interpreted by the software of used instrument by the crossing (or not crossing) of the fluorescence curve with the threshold line.

Results are accepted as relevant if both positive and negative controls of amplification along with negative control of extraction are passed.

Results for controls

Control	Stage for control	Ct channel Green/FAM	Ct channel Yellow/HEX	Ct channel Orange/ROX	Interpretation
C-	RNA/DNA extraction, Amplification	Pos	Neg	Neg	OK
PCE	RNA/DNA extraction, Amplification	Pos	Pos	Pos	OK
C+$_{HBV\ /\ HDV\text{-}FL}$	Amplification	Pos	Pos	Pos	OK
NCA	Amplification	Neg	Neg	Neg	OK

For Ct values see **Important product information bulletin**.

1. The sample is considered to be positive for *HBV* DNA if its Ct value is defined in the results grid in the JOE/HEX/Yellow channel and if it does not exceed the threshold Ct value.

2. The sample is considered to be negative for *HBV* DNA if its Ct value is not defined in the results grid (the fluorescence curve does not cross the threshold line) in the JOE/HEX/Yellow channel or if it exceeds the threshold Ct value and the Ct value in the results grid in the IC channel does not exceed the threshold Ct value.
3. The sample is considered to be positive for *HDV* RNA if its Ct value is defined in the results grid in the ROX/Orange channel and if it does not exceed the threshold Ct value.
4. The sample is considered to be negative for *HDV* RNA if its Ct value is not defined in the results grid (the fluorescence curve does not cross the threshold line) in the ROX/Orange channel or if it exceeds the threshold Ct value and the Ct value in the results grid in the IC channel does not exceed the threshold Ct value.
5. The sample is considered to be equivocal in case of equivocal result in any channel. The PCR analysis is recommended to be repeated.

10. TROUBLESHOOTING

Results of analysis are not being registered in the following cases:

1. If for Positive Controls (C+$_{HBV\ /\ HDV\text{-FL}}$ and PCE) the Ct value exceeds the threshold Ct value in the HEX/Yellow, the analysis of samples which contained no *HBV* DNA should be repeated starting from the extraction stage.
2. If for Positive Controls (C+$_{HBV\ /\ HDV\text{-FL}}$ and PCE) the Ct value exceeds the threshold Ct value in the ROX/Orange, the analysis of samples which contained no *HDV* RNA should be repeated starting from the extraction stage.
3. If for negative Controls (C- and NCA) the Ct value doesn't exceed the threshold Ct value in the HEX/Yellow or ROX/Orange, the PCR of samples which contained *HBV* DNA or *HDV* RNA should be repeated starting from the extraction stage.

11. TRANSPORTATION

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

Positive Control cDNA *HBV / HDV*-FL, Positive Control *HBV / HDV*-rec, and Internal Control *ICZ*-rec should not be frozen/thawed more than twice. After thawing, Positive Control cDNA *HBV / HDV*-FL, Positive Control *HBV / HDV*-rec, and Internal Control *ICZ*-rec are to be stored at 2-8 °C for up to 6 months.

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

13. SPECIFICATIONS

13.1. Sensitivity

Analytical Sensitivity of **AmpliSens® *HBV / HDV*-FRT** PCR kit is given the table below.

Isolation volume, µl	RNA/DNA extraction kit	Analytical sensitivity	
		***HBV*, ME/ml**	***HDV*, copies/ml**
100	RIBO-sorb RIBO-prep NucliSENS easyMAG	100	100
200	MAGNO-sorb	50	50
1000	MAGNO-sorb NucliSENS easyMAG	10	10

13.2. Specificity

The analytical specificity of **AmpliSens® *HBV / HDV*-FRT** 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 B 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, S.agalactiae;* and *Homo sapiens*. Cross reactions for marked organisms and viruses are not registered. Cross- reaction for indicated organisms and viruses were not registered. The clinical specificity of **AmpliSens® *HBV / HDV*-FRT** 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.
2. Guidelines “Real-time PCR detection of simultaneous detection of hepatitis virus B (*HBV)* DNA and hepatitis virus D (*HDV)* RNA in the clinical materials”, 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.

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 / HDV*-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
PCE	Positive Control of Extraction	**C+*HBV / HDV*-FL**	Positive Control of Amplification
FBIS CRIE	Federal Budget Institute of Science “Central Research Institute for Epidemiology”	**IC**	Internal control

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
29.11.10	Footer	Catalogue number R-V56-(RG,iQ,Mx,Dt) is changed into R-V56(RG,iQ,Mx,Dt)-CE
30.11.10	Sampling and handling	Sentence «Blood samples are taken after overnight fasting into tubes with 3% EDTA solution (1 : 20)» is changed into «Blood samples are taken after overnight fasting into the tube with EDTA solution as anticoagulant»
	Through the text	MAGNO-sorb mention was deleted
		Corrections through the text
		Abreviation C+$_{HBV/HDV\text{-FL}}$ is added for Positive Control cDNA *HBV* / *HDV*-FL
21.03.11 RT	Stability and storage	The phrase about keeping away from light of RT-PCR-mix-1-FL *HBV* / *HDV* was added
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
	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. RNA isolation	The information about using RIBO-prep kit was added
21.06.12 BO	Cover Page	IVD-symbol was changed to RUO
	Text	“tips with aerosol barriers” was changed to “tips with filters”
		Isolation was changed to extraction
	Sensitivity	Sensitivity for sample 200 µl was added
	8.1. RNA Extraction	Information about MAGNO-sorb was added
10.07.13 FN	Content	The second form was added – PCR kit variant FRT in bulk
	Footer	**REF** R-V56(RG,iQ,Mx,Dt)-CE-B was added