

IVD For *in Vitro* Diagnostic Use

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AmpliSens[®] HBV/HDV-FRT PCR kit
Instruction Manual

AmpliSens[®]

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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 virus B (*HBV*) DNA and hepatitis virus D (*HDV*) RNA 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 virus B (*HBV*) DNA and hepatitis virus D (*HDV*) RNA are isolated 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 isolation procedure in order to control the isolation 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 1 form:

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

R-V56(RG,iQ,Mx,Dt)-E.

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 <i>HBV/HDV</i>	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 (MMIv)	colorless, clear liquid	0.01	4 tubes
Positive Control cDNA <i>HBV/HDV-FL (C+)</i>	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 <i>HBV/HDV-rec**</i>	colorless, clear liquid	0.06	4 tubes
Internal Control <i>ICZ-rec***</i>	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 isolation kit
- Disposable powder-free gloves and laboratory coat
- Automated pipettors (dosers) of variable volumes
- Sterile RNase/DNase-free pipette tips with aerosol barriers (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 (BioRad, USA), Mx3000P (Stratagene, USA)) or equivalent)
- Disposable polypropylene microtubes for PCR with 0.2 or 0.1 ml capacity (for example, “Axygen”, USA)
- Refrigerator for temperature between 2 and 8 °C
- Deep-freezer with temperature not more than 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 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 is 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 isolation kits from peripheral blood plasma.

- *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 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. PROTOCOL

7.1. RNA Isolation

It's 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-E
- Automatic device "NucliSENS® easyMAG™" can also be used.



Carry out the RNA/DNA isolation 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 isolation depends on the reagents kit used:

- add 10 µl of Internal Control ICZ-rec to the sample/lysis mixture ("RIBO-prep" or "RIBO-sorb")
- add 0.28 ml of Internal Control ICZ-rec to the lysis mixture ("MAGNO-sorb", 24-tube panel extraction)
- add Internal Control ICZ-rec as specified in the manufacturer manual ("MAGNO-sorb", isolation from less than 24 samples)



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 "NucliSENS® easyMAG™" automated system is applied:

- use of "EM-plus" kit **REF** K2-15-96 (manufactured by 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 isolation 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.

7.2. Preparing the PCR

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

7.2.1 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 (MMIv)** 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 aerosol barrier add **10 µl** of **RNA/DNA** obtained from clinical samples.



When adding of RNA/DNA samples isolated by “RIBO-sorb”, “MAGNO-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** isolated from **Positive Control HBV/HDV-rec** sample to the tube labeled NCA (Positive Control of Extraction).
- C-** - Add **10 µl** of **RNA/DNA sample** isolated from **Negative Control** sample to the tube labeled C- (Negative Control of Extraction).
- C+** - Add **10 µl** of **Positive Control cDNA HBV/HDV-FL** to the tube labeled C+ (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).

7.2. 2. Amplification

7.2.2.1. 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.

7.2.2.2. 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.

8. 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 isolation, Amplification	Pos	Neg	Neg	OK
PCE	RNA/DNA isolation, Amplification	Pos	Pos	Pos	OK
C+	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.

9. TROUBLESHOOTING

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

1. If for Positive Controls (C+ 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+ 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.

10. STABILITY AND STORAGE

All components of the **AmpliSens® HBV/HDV-FRT** PCR are to be stored at the temperature not more than minus 16°C when not in use. All components of the **AmpliSens® HBV/HDV-FRT** PCR kit are stable until the labeled expiration date.



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* should be stored from 2 to 8°C for up to 6 months.

11. SPECIFICATIONS

11.1. Sensitivity

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

Isolation volume, µl	RNA/DNA isolation kit	Analytical sensitivity	
		<i>HBV</i> , ME/ml	<i>HDV</i> , copies/ml
100	"RIBO-sorb" "RIBO-prep" "NucliSENS easyMAG"	100	100
200	"MAGNO-sorb"	50	50
1000	"MAGNO-sorb" "NucliSENS easyMAG"	10	10

11.2. Specificity

The analytical specificity of **AmpliSens® HBV/HDV-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 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*; *Streptococcus 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












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 "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 State Institution of Science Central Research Institute of Epidemiology of Federal Service for Surveillance on Consumers' Rights Protection and Human Well-Being, Moscow.

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/HDV-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		Authorised representative in the European Community.
	Contains sufficient for <n> tests		Caution, consult accompanying documents
	Consult instructions for use	IC	Internal Control
C+	Positive Control of Amplification	NCA	Negative Control of Amplification
PCE	Positive Control of Extraction	C-	Negative control of Extraction
CRIE	Central Research Institute of Epidemiology (Moscow, Russia)		