

For *in Vitro* Diagnostic Use

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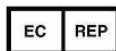
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# AmpliSens® HDV-FRT PCR kit

## Instruction Manual



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## 1. INTENDED USE

**AmpliSens® HDV-FRT** PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of 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 D virus (*HDV*) RNA is isolated from blood plasma together with internal control sample (IC). *HDV* detection by the polymerase chain reaction (PCR) is based on the reverse transcription of RNA and amplification of pathogen genome specific region using special *HDV* 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® 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® 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 *HDV* cDNA amplification product is detected in the JOE channel. The Positive Control of Extraction, Positive Control *HDV*-rec, is detected in FAM (IC) and JOE (*HDV*) channels. The Positive Control of Amplification, Positive Control cDNA *HDV*-FL, is the complex control for *HDV* and IC. It is detected in FAM (IC) and JOE (*HDV*) channels.

## 3. CONTENT

**AmpliSens® HDV-FRT** PCR kit is produced in 1 form:

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

**REF** R-V3(RG,iQ,Mx,Dt)-CE.

**AmpliSens® 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>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>HDV</i> -FL (C+ <i>HDV</i> -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 <i>HDV</i> -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® 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 (Bio-Rad, USA), Mx3000P (Stratagene, USA) or equivalent).
- Disposable polypropylene microtubes for PCR with 0.2 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 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® 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 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. PROTOCOL

### 7.1. RNA extraction

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

**REF** R-V3(RG,iQ,Mx,Dt)-CE / **VER** 18.10.10 – 04.12.10 /Page 5 of 11

- RIBO-sorb, **REF** K2-1-Et-50-CE (2 kits)
- RIBO-prep, **REF** K2-9-Et-50-CE (2 kits)
- Automated system 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-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 a 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 NucliSENS easyMAG automated system is applied:

- use of EM-plus kit **REF** K2-15-96 (manufactured by CRIE) is obligatory
- 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 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.

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- To prepare the reaction mix, mix reagents **10 µl of RT-PCR-mix-1-FL 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.
- Transfer **15 µl** of prepared mix into each tube.
- Using tips with aerosol barrier add **10 µl of RNA** obtained from clinical samples.



When adding of RNA samples isolated by RIBO-sorb and NucliSENS easyMAG it is necessary to avoid transferring of the sorbent into the reaction mix.

- Carry out the control amplification reactions:

- PCE** - Add **10 µl** of **RNA sample** isolated from **Positive Control HDV-rec** sample to the tube labeled NCA (Positive Control of Extraction).
- C-** - Add **10 µl** of **RNA sample** isolated from **Negative Control** sample to the tube labeled C- (Negative Control of Extraction).
- C+<sub>HDV-FL</sub>** - Add **10 µl** of **Positive Control cDNA HDV-FL** to the tube labeled C+<sub>HDV-FL</sub> (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

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

**AmpliSens-1 RG program**

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



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



ROX/Orange and Cy5/Red are switched on if needs for “multiprime” format tests.

- Fluorescence detection is on the 2-nd pass (**60 °C**) in FAM/Green and JOE/Yellow fluorometer channels.
- Make the adjustment of the fluorescence channel sensitivity according to guidelines.

### 7.2.2.2. iQ5

- Program the iQ according to manufacturer’s manual and guidelines.
- 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 sec	–	5
	60	20 sec	–	
	72	15 sec	–	
4	95	5 sec	–	40
	60	30 sec	FAM, HEX, ROX, Cy5	
	72	15 sec	–	



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



ROX/Orange and Cy5/Red are switched on if needs for “multiprime” format tests.

- Fluorescence detection is on the 2-nd pass (**60 °C**) in FAM and HEX fluorometer channels.
- Make the adjustment of the fluorescence channel sensitivity according to guidelines.

## 8. DATA ANALYSIS

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

See guidelines for data analysis settings for Rotor-Gene 3000 or Rotor-Gene 6000 and for iQ5.

### Results interpretation

The results are interpreted by the software of Rotor-Gene 3000 or Rotor-Gene 6000 or iQ5 Instrument by the crossing (or not) 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 in channel		Interpretation
		Green/FAM	Yellow/HEX	
<b>C-</b>	RNA isolation	Pos	Neg	OK
<b>PCE</b>	RNA isolation	Pos	Pos	OK
<b>C+<sub>HDV-FL</sub></b>	Amplification	Pos	Pos	OK
<b>NCA</b>	Amplification	Neg	Neg	OK

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

1. The sample is considered to be positive for *HDV* RNA if its Ct value is defined in the results grid in JOE/HEX/Yellow channel and if it doesn't exceed threshold Ct value.
2. 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 JOE/HEX/Yellow channel or if it exceeds threshold Ct value and in the results grid in the IC channel the Ct value doesn't exceed threshold Ct value.
3. 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<sup>+</sup><sub>HDV-FL</sub> and PCE) the Ct value exceeds the threshold Ct value in HEX/Yellow, the analysis of samples which contained no RNA *HDV* should be repeated from the extraction stage.
2. If for negative Controls (C- and NCA) the Ct value doesn't exceed the threshold Ct value in HEX/Yellow, the PCR of samples which contained RNA *HDV* should be repeated from the extraction stage.

## 10. STABILITY AND STORAGE

All components of the **AmpliSens<sup>®</sup> HDV-FRT** PCR are to be stored at ≤ -16 °C when not in use. All components of the **AmpliSens<sup>®</sup> HDV-FRT** PCR kit are to be stable until labeled expiration date.



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

## 11. SPECIFICATIONS

### 11.1. Sensitivity

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

Isolation volume, µl	RNA/DNA isolation kit	Analytical sensitivity, copies/ml
100	RIBO-sorb RIBO-prep NucliSENS easyMAG	100
1000	NucliSENS easyMAG	10



The claimed analytical features of **AmpliSens<sup>®</sup> HDV-FRT** PCR kit are guaranteed only when additional reagents kits RIBO-sorb or RIBO-prep (manufactured by Federal State Institution of Science Central Research Institute of Epidemiology) are used. NucliSENS easyMAG manufactured by bioMérieux, France can be used either.

### 11.2. Specificity

The analytical specificity of **AmpliSens<sup>®</sup> 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; 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*. Cross reactions for marked organisms and viruses are not registered. The clinical specificity of **AmpliSens<sup>®</sup> 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 hepatitis virus B, C or D RNA/DNA", 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<sup>®</sup> 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		Central Research Institute of Epidemiology (Moscow, Russia)
	Contains sufficient for <n> tests		Caution, consult accompanying documents
	Consult instructions for use		Internal Control
	Positive Control of Amplification		Negative Control of Amplification
	Positive Control of Extraction		Negative control of Extraction