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For Professional Use Only

AmpliSens® *HGV*-FRT PCR kit

Instruction Manual

AmpliSens®

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1. INTENDED USE

AmpliSens® *HGV*-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *hepatitis G virus* (*HGV)* 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

HGV detection by the polymerase chain reaction (PCR) is based on the *HGV* RNA extraction from blood plasma together with the internal control sample (IC); the reverse transcription of *HGV* RNA and the amplification of the pathogen genome specific region using special *HGV* primers. In real-time PCR, the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes that bind specifically to the amplified product during thermocycling. The real-time monitoring of fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run. The **AmpliSens® *HGV*-FRT** PCR kit is a qualitative test that contains 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. The **AmpliSens® *HGV*-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 a chemically modified polymerase (TaqF). The 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 *HGV* cDNA amplification product is detected in the JOE channel. The Positive Control of Extraction, Positive Control *HGV*-FL-rec, is detected in FAM (IC) and JOE (*HGV*) channels. The Positive Control of Amplification, Positive Control cDNA *HGV*-FL, is a complex control for *HGV* and IC. It is detected in FAM (IC) and JOE (*HGV*) channels.

3. CONTENT

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

AmpliSens® *HGV*-FRT PCR kit variant FRT-50 F (for use with RG, iQ, Mx, Dt)

REF R-V2-50-F(RG,iQ,Mx,Dt)-CE.

AmpliSens® *HGV*-FRT PCR kit variant FRT-50 F includes:

Reagent	*Description*	*Volume, ml*	*Quantity*
RT-G-mix-2	colorless clear liquid	0.015	1 tube
RT-PCR-mix-1-FL *HGV*	colorless clear liquid	0.6	1 tube
RT-PCR-mix-2-FEP/FRT	colorless clear liquid	0.3	1 tube
Polymerase (TaqF)	colorless clear liquid	0.03	1 tube
TM-Revertase (MMlv)	colorless clear liquid	0.015	1 tube
Positive Control cDNA *HGV*-FL (C+$_{HGV\text{-}FL}$)	colorless clear liquid	0.1	1 tube
Buffer for elution	colorless clear liquid	1.2	1 tube
Negative Control (C–)*	colorless clear liquid	1.2	1 tube
Positive Control *HGV*-FL-rec**	colorless clear liquid	0.1	1 tube
Internal Control *ICZ*-rec (IC)***	colorless clear liquid	0.28	2 tubes

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

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

***Add 10 µl of Internal Control *ICZ*-rec (IC) during the RNA extraction procedure directly to the sample/lysis solution before the extraction.

AmpliSens® *HGV*-FRT PCR kit is intended for 55 reactions, including controls.

4. ADDITIONAL REQUIREMENTS

- RNA/DNA extraction kit.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile RNase/DNase-free pipette tips with aerosol barriers (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 2-ml reaction tubes.
- PCR box.
- Personal thermocyclers (for example, Rotor-Gene 3000 or Rotor-Gene 6000 (Corbett Research, Australia); iCycler iQ5 (Bio-Rad, USA), Mx3000P (Stratagene, USA) or those recommended in the Guidelines [2] to the PCR-kit).
- Disposable polypropylene tubes for PCR (0.2 ml; 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 another 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

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® *HGV*-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 (serum).

- *Peripheral blood plasma (serum)*.

Blood samples are taken into the tube with 3% EDTA solution (20 parts of blood to 1 part of EDTA). 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. After that blood plasma should be taken and transferred to new disposable tubes.

To obtain serum, tubes with blood should be incubated at room temperature to allow complete clot formation. Then tubes are centrifuged at 800–1600 g for 10 min. After that serum should be taken and transferred to new disposable tubes.

Blood plasma (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® *HGV*-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-prep, REF K2-9-Et-50-CE.
- Automated system NucliSENS easyMAG can also be used.

Extract RNA according to the manufacturer's instructions.

For Positive Control of extraction (PCE) mix 10 µl of Positive Control *HGV*-FL-rec and 90 µl of Negative Control (C–)

Volume of Internal Control added during RNA extraction depends on the reagent kit used:

- add 10 µl of Internal Control *ICZ*-rec to a sample/lysis solution (RIBO-prep)

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 13,000 rpm.

It is possible to mix in a new sterile vial the Solution for Lysis and IC (300 µl of Solution for Lysis and 10 µl of IC per 1 sample). Then, 300 µl of the prepared mixture should be transferred to the 1.5-ml disposable tubes.

If *HGV* RNA, *HDV* RNA, *HCV* RNA, *HBV* DNA, *HIV* RNA and RNA for *HCV*-typing are extracted simultaneously, add all required Internal controls.

If NucliSENS easyMAG automated system is used:

- The use of EM-plus kit REF K2-15-96-CE (manufactured by CRIE) is obligatory.
- Add 30 ml (the whole content of the vial) of the **RT-G component from the EM-Plus kit** to the vial 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 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 [2] for details.

The purified RNA can be stored at 2–8 °C for 4 hours, at temperatures not more than minus 16 °C for one month or at temperatures not more than minus 68 °C for one year.

8.2. Preparing the PCR

Total reaction volume is **25 µl**, the volume of RNA 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 two controls of amplification). The type of tubes depends on the PCR instrument used for analysis.
3. To prepare the reaction mixture, mix reagents per one reaction in a new sterile tube: **10 µl of RT-PCR-mix-1-FL *HGV*, 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)**. Thoroughly vortex the

mixture, make sure that there are no drops on the caps of the tubes.

4. Transfer **15 µl** of the prepared mixture into each tube.
5. Add **10 µl** of **RNA** obtained from clinical samples into the prepared tubes using tips with aerosol barrier.

When adding of RNA samples extracted by NucliSENS easyMAG it is necessary to avoid transferring of the sorbent into the reaction mix.

6. Carry out the control amplification reactions:

PCE - Add **10 µl** of **RNA sample** extracted from the **Positive Control *HGV*-FL-rec** sample to the tube labeled PCE (Positive Control of Extraction).

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

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

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

8.2.2. Amplification

Program the real-time amplification instrument according to manufacturer's manual and the Guidelines [2].

1. Create a temperature profile on your instrument as follows:

Table 1

AmpliSens-2 RG program for rotor-type instruments[1]

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

[1] For example, Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q, or recommended in the Guidelines [2].

Table 2

AmpliSens-2 iQ program for plate-type instruments[2]

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, JOE/HEX, ROX, Cy5	
	72	15 sec	–	

AmpliSens-2 RG and **AmpliSens-2 iQ** general programs allow simultaneous conducting of tests for *HGV* detection with *HBV*, *HCV*, *HDV*, *HCV* typing or others

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

2. Fluorescent signal is detected in the channels designed for the FAM/Green and JOE/HEX/Yellow channels on the 2nd step (60 °C) of stage Cycling 2.
3. Adjust the fluorescence channel sensitivity according to the Guidelines [2] and the *Important Product Information Bulletin*.
4. Insert tubes into the reaction module of the device.
5. Run the amplification program with fluorescence detection.
6. Analyze results after the amplification program is completed.

9. DATA ANALYSIS

Internal Control is detected in the FAM/Green fluorescence channel, *HGV* cDNA is detected in the JOE/HEX/Yellow fluorescence channel.

See the Guidelines [2] for data analysis settings for Rotor-Gene 3000/6000, Rotor-Gene Q and for iCycler iQ5 and Mx3000P.

9.1. Interpretation of results

The results are interpreted by the software of Rotor-Gene 3000/6000, Rotor-Gene Q, iCycler iQ5 or Mx3000P Instrument by the crossing (or not-crossing) of the fluorescence curve with the threshold line.

The results of analysis are considered reliable only if the results obtained for both Positive and Negative controls of amplification as well as for the Positive and Negative

[2] For example, iCycler iQ5, Mx3000P, or those recommended in the Guidelines [2].

controls of extraction are correct.

Table 3

Results for controls

Control	Stage for control	*Ct* value in channel		Interpretation
		FAM/Green	JOE/Yellow/HEX	
C–	RNA extraction, Amplification	< boundary *Ct* value	*Ct* value is absent	OK
PCE	RNA extraction, Amplification	< boundary *Ct* value	< boundary Ct value	OK
C+$_{HGV\text{-}FL}$	Amplification	< boundary *Ct* value	< boundary Ct value	OK
NCA	Amplification	*Ct* value is absent	*Ct* value is absent	OK

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

1. The sample is considered to be positive for *HGV* RNA if its *Ct* value is determined in the results grid in the JOE/HEX/Yellow channel and if it does not exceed the specified boundary *Ct* value.
2. The sample is considered to be negative for *HGV* RNA if its *Ct* value is not determined in the results grid (the fluorescence curve does not cross the threshold line) in JOE/HEX/Yellow channel or if it exceeds the specified boundary *Ct* value; and in the results grid in the IC channel the threshold *Ct* value does not exceed specified boundary *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.

10. TROUBLESHOOTING

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

1. If the *Ct* value is absent or it exceeds the specified boundary Ct value for the positive control of extraction (PCE) or the positive control of amplification (C+$_{HGV\text{-}FL}$) in the JOE/Yellow/HEX channel, the analysis of samples in which *HGV* RNA was not detected should be repeated starting from the RNA extraction stage.
2. If a *Ct* value is present for negative controls of extraction (C–) and/or the negative control of amplification (NCA) in the JOE/Yellow/HEX channel, the analysis of samples in which *HGV* RNA was determined; should be repeated from the RNA extraction stage.

11. TRANSPORTATION

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

12. STABILITY AND STORAGE

All components of the **AmpliSens® *HGV*-FRT** PCR kit are to be stored at temperature from minus 24 to minus 16 °C when not in use. All components of the **AmpliSens® *HGV*-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 *HGV*-FL, Positive Control *HGV*-FL-rec, and Internal Control *ICZ*-rec should not be frozen/thawed more than twice. After thawing, Positive Control cDNA *HGV*-FL, Positive Control *HGV*-FL-rec, and Internal Control *ICZ*-rec should be stored at 2–8 °C for up to 6 months.

RT-PCR-mix-1-FL *HGV* is to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

The analytical sensitivity of **AmpliSens® *HGV*-FRT** PCR kit is given the table below.

Table 4

Extraction volume, µl	RNA/DNA extraction kit	Analytical sensitivity, copies/ml
100	RIBO-prep NucliSENS easyMAG	500
1000	NucliSENS easyMAG	50

The claimed analytical performance characteristics of **AmpliSens® *HGV*-FRT** PCR kit are guaranteed only when additional reagent kit RIBO-prep (manufactured by FBIS CRIE) is used. NucliSENS easyMAG manufactured by bioMérieux, France can be used either.

13.2. Specificity

The analytical specificity of **AmpliSens® *HGV*-FRT** PCR kit is ensured by selection of specific primers and probes as well as by selection 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 by addition of genomic DNA/RNA of the following organisms and viruses into the reaction: H*epatitis A virus; Hepatitis B virus; Hepatitis C virus; Hepatitis D virus, Hepatitis E virus, Human immunodeficiency virus; Cytomegalovirus; Epstein-Barr virus; Herpes simplex virus* types 1 and 2; *Enterovirus* (*Coxsakie* B1, B2, B3, B4, B5, B6, *Polio* I, II, III); *Human rotavirus WA*, *Astrovirus*, *Human herpes virus* types 6 and 8; *Adenovirus* types 2, 3, and 7; and *Homo sapiens*. Cross reactions for marked organisms and viruses are not registered. The clinical specificity of **AmpliSens® *HGV*-FRT** PCR kit was confirmed in laboratory clinical trials.

14. REFERENCES

1. Handbook “Sampling, Transportation and Storage of Clinical Material for PCR Diagnostics”, developed by Federal State Institute 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*-FRT, AmpliSens® *HDV*-FRT, AmpliSens® *HBV*-FRT, and AmpliSens® *HGV*-FRT PCR kits, developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology”.

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® *HGV*-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
FBIS CRIE	Federal Budget Institute of Science “Central Research Institute for Epidemiology”	**C+*HGV*-FL**	Positive control of amplification
PCE	Positive control of extraction	**IC**	Internal control

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
15.05.12 Ivl	Title page, Key to symbols used	Symbol IVD *in vitro* diagnostic medical device was changed to RUO research use only