RUO

For Professional Use Only

AmpliSens® *HCV*-FEP

PCR kit

Instruction Manual

AmpliSens®

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

TABLE OF CONTENTS

1. INTENDED USE

AmpliSens® *HCV*-FEP PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of hepatitis C virus RNA (*HCV*) in clinical material (blood plasma) by means of end-point hybridization-fluorescence detection of amplified products.

The results of PCR analysis are taken into account in complex diagnostics of disease.

2. PRINCIPLE OF PCR DETECTION

Hepatitis C virus detection includes RNA/DNA extraction from blood plasma together with internal control sample (IC), reverse transcription of RNA, and end-point PCR amplification of DNA/cDNA. In end-point PCR the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes which bind specifically to the amplified product. Multichannel rotor-type fluorometer is specially designed to detect fluorescent excitation from the fluorophores in a reaction mix after PCR. **F**luorescent **E**nd-**P**oint PCR (FEP-PCR) allows the detection of accumulating product without re-opening of the reaction tubes after the PCR run. **AmpliSens® *HCV*-FEP** 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) activates by heating at 95ºC for 15 min.

The Internal Control is detected in the FAM channel. The *HCV* cDNA amplification product is detected in the JOE/HEX channel.

The Positive Control of Extraction, Positive Control-1-*HCV,* is detected in FAM (IC) and JOE/HEX (*HCV*) channels.

DNA calibrator PIC2 *HCV* is a complex control for *HCV* and IC. It is detected in FAM (IC) and JOE/HEX (*HCV*) channels.

3. CONTENT

AmpliSens® *HCV*-FEP is produced in 1 form:

AmpliSens® *HCV*-FEP PCR kit variant FEP, REF V1-FEP-CE.

AmpliSens® *HCV*-FEP PCR kit variant FEP includes:

Reagent	***Description***	***Volume, ml***	***Quantity***
RT-G-mix-3	colorless, clear liquid	0.015	4 tubes
RT-PCR-mix-1-FL *HCV*	colorless, clear liquid	0.3	4 tubes
RT-PCR-mix-2-FEP/FRT	colorless, clear liquid	0.2	4 tubes

Mineral oil for PCR	colorless viscous liquid	4.0	1 vial
Polymerase (TaqF)	colorless, clear liquid	0.02	4 tubes
TM-Revertase (MMlv)	colorless, clear liquid	0.01	4 tubes
DNA calibrator PIC2 *HCV**	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-1-*HCV****	colorless, clear liquid	0.06	4 tubes
Internal Control *ICZ*-rec (IC)****	colorless, clear liquid	0.28	4 tubes

* Serves as a Positive Control of Amplification (C+).

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

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

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

AmpliSens® *HCV*-FEP PCR kit variant FEP is intended for 112 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, Gradient Palm Cycler (Corbett Research, Australia), GeneAmp PCR System 2700 (Applied Biosystems, USA), Terzik (DNA-Technology, Russia).
- Fluorometer ALA-1/4 (Biosan, Latvia) or equivalent instrument.
- Disposable polypropylene microtubes for PCR with 0.5 (0.2) ml capacity (for example, Axygen, USA).
- Refrigerator with temperature between 2 and 8 °C
- Deep-freezer with temperature at or below 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 extracted positive material (samples, controls and amplicons) away from all other reagents and add it to the reaction mix in a separate area.
- Thaw all components thoroughly at room temperature before starting detection.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterwards.
- 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 specimens and unused reagents in accordance with local regulations.
- Specimens 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 mucous membranes. If skin, eyes, or mucous membranes contact, immediately flush with water and 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 of 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® ***HCV*-FEP** PCR kit is intended to analyze RNA extracted with RNA/DNA 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. WORKING CONDITIONS

AmpliSens® *HCV*-FEP PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. RNA/DNA extraction

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

- RIBO-sorb, REF K2-1-Et-100-CE ;
- RIBO-prep, REF K2-9-Et-100-CE ;
- MAGNO-sorb, REF K2-16-1000-CE;
- NucliSENS easyMAG automated system can be used as well.

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

For Positive Control of extraction (PCE) mix **10 µl Positive Control-1-*HCV*** 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 a sample/lysis mixture (RIBO-prep or RIBO-sorb)
- add **0.28 ml** of Internal Control *ICZ*-rec to a lysis mixture (MAGNO-sorb, 24-tube panel extraction)
- add Internal Control *ICZ*-rec as specified in the manufacturer manual (MAGNO-sorb, extraction 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 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 Positive Control of extraction, **PCE**, add **90 µl** of the **Negative Control (C–)** sample and **10 µl** of the **Positive Control-1-*HCV*** sample to a tube containing **lysis solution**.
- 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 used:

- the 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 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 reverse transcription and PCR

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

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

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, control (including 2 controls of extraction and 1 control of amplification), and the Background samples.
3. In case of Background tubes preparation, make the reaction mixture as follows (calculating per **one reaction**):
   - **10 µl of RT-PCR-mix-1-FL *HCV***
   - **5 µl of RT-PCR-mix-2-FEP/FRT**
   - **0.25 µl of RT-G-mix-3**

Carefully vortex prepared mixture; make sure that there are no drops on the walls of the tubes. See Appendix 1, part A as well.

4. Mark two tubes as **Background** and add **15 µl of prepared mixture** (without Polymerase (TaqF) and TM-Revertase (MMlv)) and **10 µl of buffer for elution** per each tube. Mix by pipeting. Add above **1** drop of **mineral oil for PCR.**

Background tubes that have once passed thermal cycling can be stored from 2 to 25 ºC for up to 1 month and used repeatedly.
Multiple use of Background samples is permitted in case of application of the same

PCR kit lot, the same extraction reagents, and the same type of PCR tubes.

5. Add following reagents to the tube with the rest of the reaction mixture (calculating per **one reaction**):
   - **0.5 µl of Polymerase (TaqF)**
   - **0.25 µl of TM-Revertase (MMlv)**

Carefully vortex prepared mix; make sure that there are no drops on the walls of the tubes. Refer also to Appendix 1, part A.

Volumes of Polymerase (TaqF) and TM-Revertase (MMlv) listed in Appendix 1, are calculated after deduction of 30 µl reaction mixture intended for two Background samples

6. In case of multiple use of the Background samples, make the reaction mixture as follows (calculating per **one reaction**):
   - **10 µl of RT-PCR-mix-1-FL *HCV***
   - **5 µl of RT-PCR-mix-2-FEP/FRT**
   - **0.25 µl of RT-G-mix-3**
   - **0.5 µl of Polymerase (TaqF)**
   - **0.25 µl of TM-Revertase (MMlv)**

   Carefully vortex prepared mixture. Make sure that there are no drops on the walls of the tubes. See Appendix 1, part B as well.
7. Transfer **15 µl** of prepared reaction mixture per each PCR tube. Add above **1** drop of **mineral oil for PCR.**
8. Add **10 µl** of **RNA samples** obtained from clinical or control samples at the stage of RNA extraction.

Avoid sorbent transferring together with the RNA sample in case of extraction by RIBO-sorb, MAGNO-sorb kits, or NucliSENS easyMAG automated system

9. Carry out **control reactions:**

PCE - Add **10 µl of RNA sample** extracted from Positive Control-1-*HCV* to the tube labeled PCE;

C– - add **10 µl** of **RNA sample** extracted from Negative Control to the tube labeled C-;

C+ - add **10 µl** of **DNA calibrator PIC2 *HCV*** to the tube labeled C+.

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).

Make sure that there are no drops on the walls of tubes, otherwise vortex tubes briefly.

8.2.2 Amplification

Run the following program on the thermocycler (see Table 1).

Table 1

Amplification program

Thermocyclers with active temperature adjustment[1]				**Thermocyclers with block temperature adjustment[2]**			
Step	Temperature, °C	Time	Cycles	Step	Temperature, °C	Time	Cycles
1	**50**	30 min	1	1	**50**	30 min	1
2	**95**	15 min	1	2	**95**	15 min	1
3	**95**	2 sec	45	3	**95**	10 sec	45
	60	10 sec			**60**	15 sec	
					72	15 sec	
4	**10**	Storage		4	**10**	Storage	

9. DATA ANALYSIS

Detection is conducted on ALA-1/4 florescence detector (Biosan, Latvia).

Please read ALA-1/4 Operating Manual before use of this kit.

Program the detector according to manufacturer's manual and Guidelines.

Intensity of fluorescent signal is detected in two channels:

– accumulation of Internal Control cDNA amplified product is detected in the FAM channel (or analogical);

– accumulation of *HCV* cDNA amplified product is detected in the HEX channel (or analogical).

Prior to detection, all settings should be entered and saved. Refer to **Guidelines** and **Important product information bulletin** for settings.

Results interpretation

1. When the analysis is complete the results are automatically displayed in the table in the manner of following indications:

   pos – positive result;

   neg – negative result;

   eq – equivocal result (signal is in grey zone);

   nd – invalid result (specific signal and IC signal are absent in the sample).

2. Principle of result interpretation:

   The value is considered **negative** if it is less than defined threshold of negative result; **positive** if it is more than defined threshold of positive result; **equivocal** if it fits between the thresholds.

   – Positive result in HEX channel indicates *HCV* RNA in the sample.

[1]For example Terzik (DNA-Technology), Gradient Palm Cycler (Corbett Research), MaxyGene (Axygen), GeneAmp PCR System 2400 (Perkin Elmer) etc.

[2] For example GeneAmp PCR System 2700 (Applied Biosystems), PTC-100 (MJ Research), T-personal (Biometra) etc.

– Result is invalid if the signal in FAM channel is less than defined negative threshold and the signal in HEX channel is less than defined threshold as well.

3. The result of the analysis is considered reliable only if both Positive and Negative Controls are passed (Table 2).

Table 2

Results for controls

Control	Stage for control	Result of automatic interpretation		Interpretation
		FAM channel (IC)	HEX channel *(HCV)*	
C–	RNA extraction	**IC+**	neg	OK
PCE	RNA extraction	**IC+**	pos	OK
C+	Amplification	**IC+**	pos	OK
NCA	Amplification	**IC-**	nd	OK

10. TROUBLESHOOTING

If analysis results are not obtained as per the following examples:

- If the signal less than the threshold of positive result is detected for PCE or C+ in the HEX channel, then the analysis (starting from the extraciton) should be repeated for all samples in which *HCV* RNA has not been found.
- If the signal more than the threshold of the positive result is detected for C- and/or NCA in the HEX channel, then the analysis (starting from the extraction) should be repeated for all samples in which *HCV* RNA has been found.
- If the **nd** result is obtained for samples except for NCA, the analysis should be repeated (starting from the isolation). The **nd** result is normal only for NCA sample.
- If the **eq** result is registered for samples, the analysis should be repeated (starting from the extraction). If the same result is obtained once again, the sample is considered positive.
- If positive signal is detected in C- and/or NCA, results of the analysis for all samples are considered invalid due to contamination. It is necessary to repeat the analysis of all tests, and also to take measures to detect and eliminate the source of contamination.

11. TRANSPORTATION

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

12. STABILITY AND STORAGE

All components of the **AmpliSens®** ***HCV*-FEP** PCR kit are to be stored at temperature from minus 24 to minus 16 °C when not in use. All components of the **AmpliSens®** ***HCV*-FEP** PCR kit are to be stable until labeled expiration date. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

DNA calibrator PIC2 *HCV*, Positive Control-1-*HCV,* and the Internal Control *ICZ*-rec should not be frozen/thawed more than twice. After thawing, DNA calibrator PIC2 *HCV*, Positive Control-1-*HCV,* and IC *ICZ*-rec should be stored at 2-8 ºC for up to 6 months.

RT-PCR-mix-1-FL *HCV* is to kept away from light

13. SPECIFICATIONS

13.1. Sensitivity

The analytical sensitivity of **AmpliSens® *HCV*-FEP** PCR kit is specified in the table below.

Volume of sample for extraction, µl	DNA isolation method	Analytical sensitivity, IU/ml
100	RIBO-sorb RIBO-prep NucliSENS easyMAG	250
200	MAGNO-sorb	125
1,000	MAGNO-sorb NucliSENS easyMAG	25

The claimed analytical features of **AmpliSens® *HCV*-FEP** PCR kit are guaranteed only when additional reagents kits, MAGNO-sorb, RIBO-sorb, or RIBO-prep (manufactured by FBIS CRIE) are used.

13.2. Specificity

The analytical specificity of **AmpliSens® *HCV*-FEP** 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; hepatitis D 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*. The clinical specificity of **AmpliSens® *HCV*-FEP** 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 to AmpliSens® *HCV*-FEP, AmpliSens® *HBV*-FEP, AmpliSens® *HDV*-FEP, and AmpliSens® *HGV*-FEP 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® *HCV*-FEP** 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+**	Positive control of amplification
		IC	Internal control

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
01.06.10	Page footer	Reference number is changed from V1-FEP to V1-FEP-CE
	Content, text	Name of Positive Control is changed from KB2 to PIC2
13.07.10	Text	Reference number is changed from V1-FEP to V1-FEP-CE
13.12.10	Through the text	Name of Positive Control of amplification is changed from Positive Control PIC2 *HCV* (C+) to DNA calibrator PIC2 *HCV*
05.07.11 LA	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 reagents before and after the first use was added
		Information that RT-PCR-mix-1-FL *HCV* is to be kept away from light 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/DNA isolation	The information about using RIBO-prep kit was added
20.06.12 LA	Cover page	Symbol IVD was replaced by RUO symbol
	16. Key to symbols used	
	13.1. Sensitivity	In the table of analytical sensitivity the unit of measurement was added: IU/ml
	8.1. DNA extraction	Reference number of MAGNO-sorb reagent kit was changed from K2-16-1000 to K2-16-1000-CE
		Information about extraction with MAGNO-sorb is added