RUO

For Professional Use Only

AmpliSens® *HCV*-genotype-EPh

PCR kit

Instruction Manual

AmpliSens®

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TABLE OF CONTENTS

1. INTENDED USE 3
2. PRINCIPLE OF PCR DETECTION 3
3. CONTENT 3
4. ADDITIONAL REQUIREMENTS 4
5. GENERAL PRECAUTIONS 5
6. SAMPLING AND HANDLING 5
7. WORKING CONDITIONS 6
8. PROTOCOL 6
9. DATA ANALYSIS 8
10. TROUBLESHOOTING 9
11. TRANSPORTATION 9
12. STABILITY AND STORAGE 9
13. SPECIFICATIONS 10
14. REFERENCES 10
15. QUALITY CONTROL 10
16. KEY TO SYMBOLS USED 11

1. INTENDED USE

AmpliSens® *HCV*-genotype-EPh PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection and differentiation of *hepatitis C virus (HCV)* genotypes 1a, 1b, 2, and 3a in clinical material (peripheral blood plasma) by using electrophoretic detection of the amplified products in agarose gel.

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

2. PRINCIPLE OF PCR DETECTION

Hepatitis C virus genotypes 1a, 1b, 2, 3a detection and differentiation by the polymerase chain reaction (PCR) is based on the amplification of the pathogen cDNA specific region using specific *hepatitis C virus* primers. AmpliSens® *HCV*-genotype-EPh 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 wax layer. Wax melts and reaction components mix only at 95 ºC.

3. CONTENT

AmpliSens® *HCV*-genotype-EPh PCR kit is produced in 3 forms:

AmpliSens® *HCV*-genotype-EPh variant 50 R (0.5-ml tubes) **REF** V1-G50-R0,5-CE;

AmpliSens® *HCV*-genotype-EPh variant 50 R (0.2-ml tubes) **REF** V1-G50-R0,2-CE;

AmpliSens® *HCV*-genotype-EPh variant 50 R in bulk[1] (0.2-ml tubes) **REF** V1-G50-R0,2-CE-B.

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

AmpliSens® *HCV*-genotype-EPh variant 50 R includes:

Reagent	*Description*	*Volume (ml)*	*Quantity*
PCR-mix-1-R *HCV* genotypes 1a/1b ready-to-use single-dose test tubes (*under wax)*	colorless clear liquid	0.01	55 tubes of 0.5 or 0.2 ml
PCR-mix-1-R *HCV* genotypes 2/3a ready-to-use single-dose test tubes (*under wax)*	colorless clear liquid	0.01	55 tubes of 0.5 or 0.2 ml
PCR-mix-2 red	red clear liquid	1.2	1 tube
Mineral oil for PCR	colorless viscous liquid	4.0	1 vial
Positive Control cDNA *HCV* genotype 1a ($C+_{1a}$)	colorless clear liquid	0.1	1 tube
Positive Control cDNA *HCV* genotype 1b ($C+_{1b}$)	colorless clear liquid	0.1	1 tube
Positive Control cDNA *HCV* genotype 2 ($C+_{2}$)	colorless clear liquid	0.1	1 tube
Positive Control cDNA *HCV* genotype 3a ($C+_{3a}$)	colorless clear liquid	0.1	1 tube
DNA-buffer	colorless clear liquid	0.5	1 tube
Negative Control (C-)*	colorless clear liquid	1.2	1 tube

* must be used in the isolation procedure as Negative Control of Extraction.

AmpliSens® *HCV*-genotype-EPh PCR kit variant 50 R is intended for 55 reactions, including controls.

4. ADDITIONAL REQUIREMENTS

- RNA isolation kit.
- Reverse transcription kit.
- Agarose gel detection kit.
- Disposable powder-free gloves.
- Pipettes (adjustable).
- Sterile RNase-free pipette tips with aerosol filters (up to 200 µl).
- Vortex mixer.
- Desktop microcentrifuge with a rotor for 2-ml reaction tubes (RCF max. 16,000 x g).
- PCR box or Biological cabinet.
- Tube racks.
- 1.5-ml polypropylene sterile tubes.
- Refrigerator for 2–8 °C.
- Deep-freezer for ≤ –16 °C.
- Waste bin for used tips.
- Permanent pen for labeling.

• Thermostat for tubes with controlled temperature for incubation at 25–100 °C.

• Personal thermocyclers (for example, Terzik (DNA-Technology, Russia), Gradient Palm Cycler (Corbett Research, Australia), MaxyGene (Axygen Scientific, USA)).

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

• Use sterile RNase-free pipette tips with aerosol 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 protective gloves, laboratory coats, protect eyes while samples and reagents handling. 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 samples and unused reagents in compliance with local authorities' requirements.

• Samples should be considered potentially infectious and handled in biological cabinet in compliance with appropriate biosafety practices.

• Clean and disinfect all sample or reagent spills with 0.5 % sodium hypochlorite solutions or another suitable disinfectant.

• Avoid contact with the skin, eyes, and mucous membranes. If skin, eyes, and 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 DNA amplification techniques.

• The laboratory process must be one-directional; it should begin in the Extraction Area and then move to the Amplification and Detection Area. Do not return samples, equipment, and reagents to the area where you carried out the previous step.

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 materials samples for PCR-analysis, transportation and storage are described in manufacturer's handbook [2]. It is recommended that this handbook is read before starting work.

AmpliSens® *HCV*-genotype-EPh PCR kit is intended for analysis of RNA extracted with RNA isolation kits from:

- *Peripheral blood plasma*

6.1. *Peripheral blood plasma.* Blood should be collected to a tube (for instance, Vacuette) with 6 % EDTA (50 µl of EDTA per 1.0 ml of blood) after overnight fasting. After the tube is filled, invert it several times to ensure proper mixing. Spin the tube at 3,000 rpm for 10 min. Remove and transfer blood plasma to a 1.5-ml tube using a tip with aerosol barrier. Plasma should be obtained within 6 h from the blood taking time.

Storage of samples:

- at 2– 8 ºC for 1 week;
- at ≤ –68 ºC for 1 year.

7. WORKING CONDITIONS

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

8. PROTOCOL

8.1. RNA Isolation

It is recommended to use the following nucleic acid extraction kit:

- RIBO-sorb, **REF** K2-1-Et-50-CE;

Extract RNA in compliance with the manufacturer protocol.
The volume of clinical sample is 100 µl.
The volume of Negative Control (C–) is 100 µl.

8.2. Reverse transcription

It is recommended to use the following kit for complementary DNA (cDNA) synthesis from RNA:

- REVERTA-L, **REF** K3-4-50-CE.

Carry out the reverse transcription in compliance with the manufacturer protocol.
The volume of RNA sample is 10 µl.

8.3. Preparing PCR

The total reaction volume is **25 µl**, the volume of cDNA sample is **5 µl**.

1. Prepare the required number of tubes with **PCR-mix-1-R *HCV* genotypes 1a/1b** and **PCR-mix-1-R *HCV* genotypes 2/3a** with wax for amplification of clinical and control samples cDNA.
2. Add **10 µl** of **PCR-mix-2 red** onto the surface of the wax layer of each tube ensuring that it does not fall under the wax and mix with PCR-mix-1-R.
3. Add above **1** drop of **mineral oil for PCR** (about **25 µl**).

4. Using tips with aerosol barrier, add **5 µl cDNA** obtained from clinical or control samples.

5. Carry out the control amplification reactions:

NCA -Add **5 µl** of **DNA-buffer** to the tube labeled NCA (Negative Control of Amplification)

C+$_{1a}$ -Add **5 µl** of **Positive Control cDNA *HCV* genotype 1a** to the tube labeled **C+$_{1a}$** (Positive Control of **PCR-mix-1-R *HCV* genotypes 1a/1b**)

C+$_{1b}$ -Add **5 µl** of **Positive Control cDNA *HCV* genotype 1b** to the tube labeled **C+$_{1b}$** (Positive Control of **PCR-mix-1-R *HCV* genotypes 1a/1b**)

C+$_{2}$ -Add **5 µl** of **Positive Control cDNA *HCV* genotype 2** to the tube labeled **C+$_{2}$** (Positive Control of **PCR-mix-1-R *HCV* genotypes 2/3a**).

C+$_{3a}$ -Add **5 µl** of **Positive Control cDNA *HCV* genotype 3a** to the tube labeled **C+$_{3a}$** (Positive Control of **PCR-mix-1-R *HCV* genotypes 2/3a**).

8.3.2 Amplification

Run the following program on the thermocycler (see Table 1). When the temperature reaches 95 ºC (pause mode), insert tubes into the cells of the thermocycler and press the button to continue.

It is recommended to sediment drops from walls of tubes by short centrifugation (1–3 s) before placing them in the thermocycler.

Table 1

Programming thermocyclers for cDNA amplification of *HCV* genotypes 1a, 1b, 2, and 3a

	Thermocyclers with active temperature adjustment:								
	GeneAmp PCR System 2400 (Perkin Elmer), Omn-E (Hybaid), Biometra, Terzik (DNA-Technology)			**GeneAmp PCR System 2700 (Applied Biosystems), Gradient Palm Cycler (Corbett Research)**			**MaxyGene (Axygen)**		
Step	Temperature	Time	Cycles	Temperature	Time	Cycles	Temperature	Time	Cycles
0	**95 °C**	pause		**95 °C**	pause		**95 °C**	pause	
1	**95 °C**	5 min	1	**95 °C**	5 min	1	**95 °C**	5 min	1
2	**95 °C**	10 s	42	**95 °C**	30 s	42	**95 °C**	30 s	42
	68 °C	10 s		**68 °C**	30 s		**67 °C**	45 s	
	72 °C	10 s		**72 °C**	30 s		**72 °C**	45 s	
3	**72 °C**	1 min	1	**72 °C**	1 min	1	**72 °C**	1 min	1
4	**4 °C**	storage		**4 °C**	storage		**10 °C**	storage	

Table 2

Programming thermocyclers for cDNA amplification of *HCV* genotypes 1a, 1b, 2, and 3a

	Thermocyclers with block temperature adjustment: **Uno-2 (Biometra), MiniCycler, PTC-100 (MJ Research)**		
Step	Temperature	Time	Cycles
0	**95 °C**	pause	
1	**95 °C**	5 min	1
2	**95 °C**	1 min	42
	68 °C	1 min	
	72 °C	1 min	
3	**72 °C**	1 min	1
4	**4 °C**	storage	

Amplification in thermocyclers with block temperature adjustment lasts for 2 h 30 min; in thermocyclers with active temperature adjustment, 1 h 50 min.

After the reaction is finished, PCR tubes must be collected and transferred to the room for PCR products analysis.

Analysis of amplification products is performed by separation of DNA fragments in agarose gel.

The amplified samples can be stored for 16 h at room temperature, for 1 week at 2–8 °C (be sure to warm the samples to room temperature before running electrophoresis).

9. DATA ANALYSIS

It is recommended to use the following detection agarose kit:

- EPh variant genotype-200, **REF** K6-200-CE.

Each gel row should necessarily include $C+_{1a}$, $C+_{1b}$, $C+_{2}$, $C+_{3a}$ controls and, if possible, include DNA molecular weight marker.

Analysis of results is based on the presence or absence of specific bands of amplified cDNA in 3 % agarose gel (agarose for high-resolution DNA electrophoresis is used). The lengths of specific amplified cDNA fragments are as follows:

- **genotype 1a – 338 bp**
- **genotype 1b – 395 bp**
- **genotype 2 – 286 bp**
- **genotype 3a – 227 bp**

Put on a protective mask or use a glass filter while watching and photographing the gel.

8.1 Interpretation of results

Table 3

Results for controls

Control	Which step of test is controlled	Specific bands in the agarose gel				Interpretation
		PCR-mix-1-R *HCV* genotypes 1a/1b		PCR-mix-1-R *HCV* genotypes 2/3a		
		338 bp	395 bp	286 bp	227 bp	
C-	RNA isolation	No	No	No	No	OK
NCA	Amplification	No	No	No	No	OK
$C+_{1a}$	Amplification	**Yes**	No	NA*	NA*	OK
$C+_{1b}$	Amplification	No	**Yes**	NA*	NA*	OK
$C+_{2}$	Amplification	NA*	NA*	**Yes**	No	OK
$C+_{3a}$	Amplification	NA*	NA*	No	**Yes**	OK

* Note that the **$C+_{1a}$** and **$C+_{1b}$** are *not* analyzed on PCR-mix-1-R *HCV* genotypes 2/3a; **$C+_{2}$** and **$C+_{3a}$** are *not* analyzed on PCR-mix-1-R *HCV* genotypes 1a/1b.

1. The sample is considered positive if one or more specific bands is present in agarose gel at the following levels:
   - 338 bp or 395 bp – for amplification with PCR-mix-1-R *HCV* genotypes 1a/1b;
   - 286 bp or 227 bp – for amplification with PCR-mix-1-R *HCV* genotypes 2/3a.

   In addition to the specific bands, fuzzy bands corresponding to primer dimers may appear in lanes below the 100-bp level.

10. TROUBLESHOOTING

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

- If the results of control samples do not correspond to those listed above (Table 3), the tests should be repeated.
- The appearance of nonspecific bands of different molecular weight in lanes may be caused by the lack of “hot start” or an inappropriate temperature regime in the thermocycler. In this case, the results of analysis are invalid.
- The appearance of specific bands in lanes corresponding to negative controls (NCA and C–) suggests contamination of reagents or samples. In such cases, the results of analysis are considered to be invalid. Analysis of all samples must be repeated and measures to detect and eliminate the source of contamination must be taken.

11. TRANSPORTATION

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

12. STABILITY AND STORAGE

All components of AmpliSens® *HCV*-genotype-EPh PCR kit should be stored at 2–8 ºC when not in use. All components of the 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.

13. SPECIFICATIONS

13.1. Sensitivity

The analytical sensitivity of **AmpliSens® *HCV*-genotype-EPh** PCR kit is the following:

Extraction volume, µl	Nucleic acid extraction kit	Sensitivity, IU/ml
100	RIBO-sorb	$1x10^4$

The claimed analytical features of AmpliSens® *HCV*-genotype-EPh PCR kit are guaranteed only when additional reagent kits RIBO-sorb, REVERTA-L, and EPh (manufactured by Federal Budget Institute of Science "Central Research Institute for Epidemiology") are used.

13.2. Specificity

The assessment of the analytical specificity of **AmpliSens® *HCV*-genotype-EPh** PCR kit showed the absence of cross-reactivity with *hepatitis C virus (HCV)* genotypes 1,2,3, *hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis D virus (HDV), hepatitis E virus (HEV), hepatitis G virus (HGV), human Immunodeficiency virus, cytomegalovirus, Epstein–Barr virus, human herpes virus* types 6 and 8, *Herpes simplex virus* types 1 and 2, and human DNA.

14. REFERENCES

1. Manual "Sampling, Transportation, and Storage of Clinical Material for PCR Diagnostics", developed by Federal Budget Institute of Science "Central Research Institute for Epidemiology", Moscow, 2008.

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*-genotype-EPh PCR kit is 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	**NCA**	Negative control of amplification
	Manufacturer	**C–**	Negative control of extraction
	Date of manufacture	**C+**	Positive control of amplification

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
10.07.10	Text	Reference numbers are changed from V1-G50-R0,2; V1-G50-R0,5 to V1-G50-R0,2-CE; V1-G50-R0,5-CE, respectively
	Page footer	
19.01.11 KM	Cover page	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
30.06.11 VV	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”
26.04.12 IvI	Title page, Key to symbols used	Symbol IVD *in vitro* diagnostic medical device was changed to RUO research use only
	Though the text	The information about analytical specificity and sensitivity was changed
12.08.14 ChA	Content	The second form was added PCR kit variant FRT in bulk
	Footer	Catalogue number was added **REF** V1-G50-R0,2-CE-B