



For *in Vitro* Diagnostic Use

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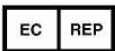
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AmpliSens[®] HCV-genotype-EPh

PCR kit

Instruction Manual

AmpliSens[®]



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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, 3a in the clinical material (peripheral blood plasma) by using electrophoretic detection of the amplified products in agarose gel.

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 pathogen cDNA specific region using special *hepatitis C virus* primers. **AmpliSens® HCV-genotype-EPh** 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 wax layer. The wax melting and reaction components mixing occur only at 95 °C.

3. CONTENTS

AmpliSens® HCV-genotype-EPh PCR kit is produced in 2 forms:

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

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

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 (<i>under wax</i>)	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 (<i>under wax</i>)	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 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 with temperature not more than –16 °C.
- Waste bin for used tips.
- Permanent pen for labeling
- Thermostat for tubes with controlled temperature and capable of incubating from 25 °C to 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 other suitable disinfectant.
- Avoid contact with the skin, eyes and mucose membranes. If skin, eyes and mucose membranes 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 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 is 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 in a tube (for instance, "Vacuette®") that contains 6 % EDTA solution (50 µl of EDTA per 1.0 ml of blood) after overnight fasting. After the tube is filled invert it several times to ensure adequate mixing. Spin the tube at 3,000 r/min for 10 min. Remove and transfer plasma in a 1.5 ml tube using aerosol filter tip. Plasma should be collected within 6 h from the time of blood taking.

Storage of samples.

- at 2 °C – 8 °C for 1 week;
- at minus 68 °C or below for 1 year.

7. PROTOCOL

7.1. RNA Isolation

It's recommended to use the following nucleic acid extraction kit:

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



Carry out the RNA isolation in compliance with the manufacturer protocol.
The volume of clinical sample is 100 µl.
The volume of Negative Control (C-) is 100 µl.

7.2. Reverse transcription

It's 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.

7.3. Preparing the PCR

Total reaction volume - **25 µl**, volume of cDNA sample - **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** to 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 filter 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**).

7.3.2 Amplification

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

It is recommended to sediment drops from walls of tubes by short vortex (1–3 sec) before their insertion in a thermocycler.

Table 1

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

Step	Thermocyclers with active temperature adjustment:								
	"GeneAmp PCR System 2400" (Perkin Elmer), "Omn-E" (Hibaid), "Biometra", "Terzik" (DNA- Technology)			"GeneAmp PCR System 2700" (Applied Biosystems), "Gradient Palm Cycler" (Corbett Research)			«Maxygene» (Axygen)		
	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 sec	42	95 °C	30 sec	42	95 °C	30 sec	42
	68 °C	10 sec		68 °C	30 sec		67 °C	45 sec	
	72 °C	10 sec		72 °C	30 sec		72 °C	45 sec	
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 1a, 1b, 2, 3a genotypes of *HCV* cDNA amplification

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 thermocycler with block temperature adjustment lasts 2 h 30 min, in thermocycler with active temperature adjustment — 1 h 50 min.

After the reaction is finished PCR tubes must be collected and sent 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).

8. DATA ANALYSIS

It's recommended to use the following detection agarose kit:

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



Each gel row should obligatory include C_{+1a}, C_{+1b}, C₊₂, C_{+3a} controls and advisory 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:

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



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

8.1 Results interpretation.

Table 3

Results for controls

Control	Which step of test is controlled	Specific bands in the agarose gel				Interpretation
		PCR-mix-1-R <i>HCV</i> genotypes 1a/1b		PCR-mix-1-R <i>HCV</i> 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 are present in agarose gel at the following levels:

- 338 bp or 395 bp – if amplifying with PCR-mix-1-R *HCV* genotypes 1a/1b;
- 286 bp or 227 bp – if amplifying with PCR-mix-1-R *HCV* genotypes 2/3a

Besides specific bands the indistinct washed-out bands of primer-dimers may be seen in lanes, they are situated lower than level of 100 bp.

9. TROUBLESHOOTING

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

- If the results of control samples do not correspond to the listed above (Table 3), then the tests are to be re-installed.
- If in lines nonspecific bands occur at different levels, it may be caused by lack of "hot start" or false temperature regimen in thermocycler.
- If in lanes corresponding to negative controls (NCA, C-) specific bands appear it means that reagents or samples contamination has taken place. In such cases analysis results must be considered as irrelevant. Test analysis must be repeated and measures for detecting contamination source must be undertaken.

10. STABILITY AND STORAGE.

All components of AmpliSens[®] *HCV*-genotype-EPh PCR kit should be stored between 2 °C and 8 °C, when not in use. They also must be stable until the expiry date stated on the label.

11. SPECIFICATIONS

11.1. Sensitivity

Analytical Sensitivity of AmpliSens[®] *HCV*-genotype-EPh PCR kit is no less than 1x10³ copies per 1 ml

of a sample (copies/ml).



The claimed analytical features of AmpliSens® HCV-genotype-EPh PCR kit are guaranteed only when additional kits of reagents, “RIBO-sorb”, “REVERTA-L”, and “EPh” (manufactured by Federal State Institution of Science Central Research Institute of Epidemiology), are used.

11.2. Specificity

Specificity of AmpliSens® HCV-genotype-EPh PCR kit is ensured by selection of specific primers and strict reaction conditions as well as laboratory and clinical trials.

12. REFERENCES

1. Manual “Sampling, transportation and 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.

13. QUALITY CONTROL

In compliance with Federal State Institution of Science Central Research Institute of Epidemiology ISO 13485 – certified Total Quality Management System, each lot of AmpliSens® HCV-genotype-EPh PCR kit is tested against predetermined specifications to ensure consistent product quality.

14. EXPLANATION OF SYMBOLS



Manufacturer



Temperature limitation



Use by



Batch code



For *in Vitro* Diagnostic Use



Version



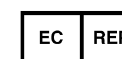
Catalogue number

ICc

Internal Control complex



Contains sufficient for <n> tests



Authorised representative in the European Community.



Consult instructions for use



Caution, consult accompanying documents

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
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