AmpliSens® *HCV*-genotype-FRT PCR kit

RUO

For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code		Consult instructions for use
RUO	Research Use Only		Contains sufficient for <n> tests
VER	Version		Use-by date
	Manufacturer	NCA	Negative control of amplification
	Date of manufacture	C–	Negative control of extraction
	Temperature limit	C+	Positive control of amplification
	Keep away from sunlight	IC	Internal control

1. INTENDED USE

AmpliSens® *HCV*-genotype-FRT PCR kit is an *in vitro* nucleic acid amplification test for differentiation of *hepatitis C virus* (*HCV*) genotypes in the biological material (peripheral blood plasma) using real-time hybridization-fluorescence detection of amplified products.
PCR kit variant FRT-g1-6 is intended to detect *HCV* genotypes 1a, 1b, 2, 3a, 4, 5a, and 6.
AmpliSens® *HCV*-genotype-FRT PCR kit is recommended for use after detection of *hepatitis C virus* RNA by **AmpliSens® *HCV*-Monitor-L** PCR kit.

NOTE: For research use only. Not for diagnostic procedures

2. PRINCIPLE OF PCR DETECTION

Detection of *HCV* genotypes 1a, 1b, 2, 3a, 4, 5a, and 6 by polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific primers. In real-time PCR, the amplified product is detected with the use of fluorescent dyes. These dyes are linked to oligonucleotide probes, which 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.
AmpliSens® *HCV*-genotype-FRT PCR kit uses "hot-start", which greatly reduces the frequency of nonspecifically primed reactions. "Hot-start" is guaranteed by the separation of nucleotides and Taq-polymerase using chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.
HCV genotype detection includes:
(a) Total RNA extraction from blood plasma simultaneously with the recombinant Internal Control (IC) sample.
(b) Reverse transcription of cDNA on RNA template.
(c) Real-time PCR of *HCV* cDNA.
To rule out possible false negative results, the Internal Control is included in the assay. This makes it possible to monitor all stages of the analysis and reveal the effect of PCR inhibitors on the result.
The PCR kit contains the system for prevention of contamination by amplicons using the enzyme uracil-DNA-glycosylase (UDG) and deoxyuridine triphosphate (dUTP).
Detection of *HCV* genotypes in a single test sample is carried out in several tubes. Either two *HCV* genotypes or *HCV* genotype and IC can be discriminated in one tube during the run.
The PCR kit is designed for the real-time PCR instruments with two and more fluorescence detection channels. The table below shows the channels for detection of *HCV* genotypes for each of the reaction mixtures used:

Table 1

Reaction mixture	**1b/3a**	**1a/2**	**IC/4**	**5a/6**
Channel for fluorophore	**Detected *HCV* genotypes**			
FAM	**1b**	**1a**	**IC**	**5a**
Target gene	Core *HCV*	Core *HCV*	Artificially synthesized sequence	NS5b *HCV*
JOE	**3a**	**2**	**4**	**6**
Target gene	Core *HCV*	5UTR+Core *HCV*	Core *HCV*	Core *HCV*

3. CONTENT

AmpliSens® *HCV*-genotype-FRT PCR kit is produced in 1 form:
variant FRT-g1-6, REF H-0256-1-3-CE.

Variant FRT-g1-6 includes:

Reagent	**Description**	**Volume, ml**	**Quantity**
PCR-mix-1-FRT *HCV* genotypes 1b/3a	clear liquid from colorless to light lilac colour	0.6	1 tube
PCR-mix-1-FRT *HCV* genotypes 1a/2	clear liquid from colorless to light lilac colour	0.6	1 tube
PCR-mix-1-FRT *HCV* IC/genotype 4	clear liquid from colorless to light lilac colour	0.6	1 tube
PCR-mix-1-FRT *HCV* genotypes 5a/6	clear liquid from colorless to light lilac colour	0.6	1 tube
PCR-buffer-C	colorless clear liquid	0.3	4 tubes
Polymerase (TaqF)	colorless clear liquid	0.03	4 tubes
Positive Control cDNA *HCV* genotypes 1b/3a (C+$_{1b/3a}$)	colorless clear liquid	0.2	1 tube
Positive Control cDNA *HCV* genotypes 1a/2 (C+$_{1a/2}$)	colorless clear liquid	0.2	1 tube
Positive Control cDNA *HCV* IC/genotype 4 (C+$_{IC/4}$)	colorless clear liquid	0.2	1 tube
Positive Control cDNA *HCV* genotypes 5a/6 (C+$_{5a/6}$)	colorless clear liquid	0.2	1 tube
TE-buffer	colorless clear liquid	0.5	1 tube
Negative Control (C–)	colorless clear liquid	1.2	1 tube
Internal Control STI-248-rec (IC)	colorless clear liquid	0.5	1 tube

Variant FRT-g1-6 is intended for 55 tests (220 amplification reactions) including controls.

4. ADDITIONAL REQUIREMENTS

- RNA extraction kit.
- Reverse transcription reagent kit.
- Pipettes (adjustable).
- Sterile RNase-free pipette tips with aerosol filters (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with a rotor for 2-ml reaction tubes.
- PCR box.
- Real-time instruments (for example, Rotor-Gene 3000 or Rotor-Gene 6000 (Corbett Research, Australia); Rotor-Gene Q (QIAGEN, Germany), CFX96 (Bio-Rad, USA), DTprime ("DNA-Technology", Russia)).
- Disposable polypropylene tubes
  a) thin-walled 0.2-ml PCR tubes with flat caps or strips of four 0.1-ml Rotor-Gene PCR tubes if a rotor-type instrument is used;
  b) thin-walled 0.2-ml PCR tubes with optical transparent domed caps or strips of eight 0.2-ml tubes with optical transparent caps if a plate-type instrument is used;
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir for used tips.
- Disposable powder-free gloves and a laboratory coat.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol filters and use a new tip for every procedure.
- Store all extracted positive material (samples, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable protective gloves and laboratory cloths, and 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 the PCR kit if the internal packaging was damaged or its appearance was changed.
- Do not use the PCR kit if the transportation and storage conditions according to the Instruction Manual were not observed.
- Do not use a kit after its expiration date.
- Dispose of all specimens and unused reagents in accordance with local regulations.
- 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 using a disinfectant, such as 0.5% sodium hypochlorite or another suitable disinfectant.
- Avoid inhalation of vapors, samples and reagents contact with the skin, eyes, and mucous membranes. Harmful if swallowed. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice if necessary.
- Safety Data Sheets (SDS) are available on request.
- The PCR kit is intended for single use for PCR analysis of specified number of samples (see the section "Content").
- The PCR kit is ready for use in accordance with the Instruction Manual. Use the PCR kit strictly for intended purpose.
- Use of this product should be limited to personnel trained in DNA amplification techniques.
- Workflow in the laboratory must be one-directional, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where 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

NOTE: Obtaining samples of biological material for PCR-analysis, transportation, and storage are described in detail in the manufacturer's handbook [1]. It is recommended that this handbook is read before starting the work.

AmpliSens® *HCV*-genotype-FRT PCR kit is intended for analysis of the RNA extracted with nucleic acid extraction kits from the biological material (peripheral blood plasma).
Collect a blood sample in a tube with 3% EDTA solution in the ratio of 20:1 (20 parts of blood to 1 part of EDTA). Invert the closed tube several times to ensure adequate mixing. Remove and transfer the plasma sample in a new tube within 6 h from the time of blood sampling. To do this, centrifuge the tube with blood at 800 – 1,600 g for 20 min.
Plasma samples can be stored:
– at 2–8 °C for up to 3 days;
– at the temperature not more than minus 68 °C for a long time.

7. WORKING CONDITIONS

AmpliSens® *HCV*-genotype-FRT PCR kit should be used at the temperature from 20 to 28 °C and relative humidity from 15 to 75 %.

8. PROTOCOL

8.1. RNA extraction

It's recommended to use the following nucleic acid extraction kits:
- RIBO-prep.

The RNA extraction of each test sample is carried out in the presence of **Internal Control STI-248-rec (IC)**.
Add 10 µl of **Internal Control STI-248-rec (IC)** to the tubes with test samples and control samples.
In the extraction procedure it is necessary to carry out the control reactions as follows:

C– – Add **Negative Control (C–)** to tube labeled **C–** (Negative Control of Extraction).

Extract RNA according to the manufacturer's protocol taking into account next additions and improvements:

NOTE:
- The volume of Negative Control (C–) is 100 µl.
- The volume of the test sample is 100 µl.
- The elution volume should be **100 µl**.

NOTE: RNase-free and DNase-free plastic consumables should be used only.

NOTE: Obtained RNA is not to be stored.
Proceed to reverse transcription immediately after RNA extraction.

8.2. Reverse transcription

It's recommended that the following reverse transcription reagent kit is used:
- **REVERTA-L** (procedure is described in Section 8.2.1).

8.2.1 Reverse transcription with REVERTA-L RT reagents kit variant 50

NOTE: RNase-free and DNase-free plastic consumables should be used only.
All components of the reaction mixture should be mixed immediately before use.

The total reaction volume is **20 µl**, the volume of RNA sample is **10 µl**.

A. Preparing tubes
1. Thaw the tubes with **RT-mix** and **RT-G-mix-1**and thoroughly vortex. Remove drops from the walls of the tubes.
2. Take the required number of 0.2- or 0.5-ml tubes (depends on the type of thermocycler or thermostat to be used) including a tube for Negative Control of extraction (C–). Mark the tubes.
3. Reverse transcription for 10-12 samples:
   a) Prepare the reaction mixture for 12 reactions. To do this, add **5 µl** of **RT-G-mix-1** to the tube with **RT-mix** and vortex. To remove drops from tubes walls, centrifuge briefly.
   b) Add **6 µl** of **revertase (MMlv)** to the tube with the reaction mixture, pipette 5 times, and vortex. To remove drops from tubes walls, centrifuge briefly.
4. Reverse transcription for less than 10 samples:
   In a new tube mix the reagents in the following order: **10 µl of RT-mix, 0.4 µl of RT-G-mix-1**, and **0.5 µl of revertase (MMlv)** (the quantities are calculated per one reaction; also see table 2). When adding RT-G-mix-1 and revertase (MMlv), pipette each reagent at least 5 times. Vortex the mixture and remove drops from the walls of the tubes.

NOTE: **Revertase (MMlv)** is temperature-sensitive and should not be kept at room temperature for a long while! Place the reagent in a freezer immediately after use.

Table 2

Scheme of reaction mixture preparation

Volume of reagent per one reaction, µl	10.0	0.4	0.5
Number of test samples	RT-mix	RT-G-mix-1	Revertase (MMlv)
4	60[1]	2.4	3.0
5	70	2.8	3.5
6	80	3.2	4.0
7	90	3.6	4.5
8	100	4.0	5.0

5. Transfer **10 µl** of the prepared mixture into each tube.
6. Add **10 µl of RNA-sample** to each tube with the reaction mixture. Carefully vortex. To remove drops from tubes walls, centrifuge briefly.

B. Reverse transcription
1. Place the tubes in a thermostat (or a thermal cycler) and incubate at 37 °C for 30 min.

NOTE: If reverse transcription is carried out with the use of a real-time thermocycler, assign the required program (see Table 3 and Guidelines [2]).

Table 3

Program for reverse transcription to be carried out in a real-time thermocycler[2]

Step	Temperature, °C	Time	Fluorescent signal detection	Cycles
1	37	30 min	–	1

2. Add **20 µl of DNA-buffer** to each tube by the end of the reverse transcription. Use a new tip for each sample. Carefully vortex the tubes. Make sure there are no drops on the walls of the tubes. Obtained cDNA samples can be used for PCR.

Storage of cDNA samples:
– at the temperature not more than minus 16 °C for 1 week;
– at the temperature not more than minus 68 °C for 1 year.

8.3 Preparing the PCR

8.3.1 Preparing the PCR

The total reaction volume is **25 µl**, the volume of the cDNA sample is **10 µl**.
The type of tubes depends on the PCR instrument used for analysis. Use disposable filter tips for adding reagents, cDNA and control samples into tubes.

NOTE: **Prepare reaction mixture just before PCR analysis. See table 4 for the scheme of reaction mixture preparation.**

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1. Thaw the reagents, thoroughly vortex the tubes, and centrifuge shortly to remove drops from the walls of the tubes.
2. Take the required number of PCR tubes (including 1 control of RNA extraction and 2 controls of amplification).

NOTE: Each sample should be analyzed with the use of 4 reaction mixtures; therefore, prepare 4 tubes for each sample. If rotor-type instrument is used, label the 0.2-ml tubes as follows:
sample no._1b/3a; sample no._1a/2; sample no._IC/4; sample no._5a/6.
If striped tubes are used for rotor-type instruments or if the analysis is carried out in a plate-type instrument, use a marked plate.

3. Collect 4 tubes of 0.5 ml to prepare the reaction mixtures. Label the tubes as follows: **1b/3a**, **1a/2**, **IC/4, and 5a/6**.
4. Per each of four labeled tubes add the following reagents (calculating per one reaction): **5 µl of PCR-buffer-C, 0.5 µl of polymerase (TaqF), 10 µl** of the required **PCR-mix-1-FRT *HCV* genotype** (see table 4). Make sure that **PCR-mix-1-FRT *HCV* genotypes 1b/3a** is added to the tubes labeled 1b/3a and so on. Vortex the tubes with prepared reaction mixtures and centrifuge shortly to remove drops from the tubes walls.

Table 4

Scheme of reaction mixture preparation

Reagent volume per one reaction, µl		10.0	5.00	0.50
Number of test samples	Number of test samples[3]	PCR-mix-1-FRT *HCV* genotype__*	PCR-buffer-C*	Polymerase (TaqF)*
4	7	80	40	4.0
5	8	90	45	4.5
6	9	100	50	5.0
7	10	110	55	5.5
8	11	120	60	6.0
9	12	130	65	6.5
10	13	140	70	7.0
11	14	150	75	7.5
12	15	160	80	8.0

* Volumes for one extra reaction are included.

5. Transfer **15 µl** of the **prepared mixture** to the PCR tubes according to marking. Make sure that PCR-mix-1-FRT *HCV* genotypes 1b/3a is added to the tubes labeled as "sample no._1b/3a" and so on.
   If striped tubes are used, the reaction mixtures should be transferred to the tubes in accordance with the order indicated on Fig. 1.

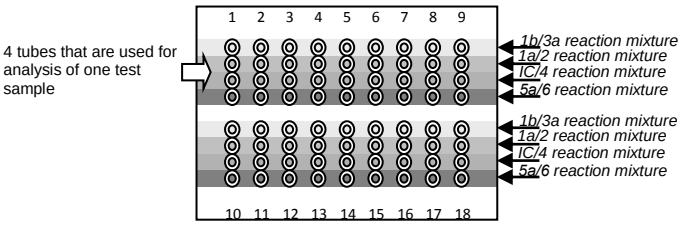

Figure 1 – Scheme of distribution of reaction mixtures and samples in case striped tubes are used.

6. Add **10 µl** of **cDNA samples** obtained at the stage of reverse transcription to the tubes. Make sure that each sample (including Negative Control of extraction, C–) is added to 4 tubes containing 1b/3a, 1a/2, IC/4, and 5a/6 reaction mixtures.
7. Carry out control amplification reactions:

NCA — Add **10 µl** of **TE-buffer** to the tubes containing **1b/3a, 1a/2, IC/4, and 5a/6** reaction mixtures (Negative Control of Amplification).
$C+_{1b/3}$ — Add **10 µl** of **Positive Control cDNA *HCV* genotypes 1b/3a ($C+_{1b/3a}$)** to the tube with **1b/3a** reaction mixture (Positive Control of Amplification).
$C+_{1a/2}$ — Add **10 µl** of **Positive Control cDNA *HCV* genotypes 1a/2 ($C+_{1a/2}$)** to the tube with **1a/2** reaction mixture (Positive Control of Amplification)
$C+_{IC/4}$ — Add **10 µl** of **Positive Control cDNA *HCV* IC/genotype 4 ($C+_{IC/4}$)** to the tube with **IC/4** reaction mixture (Positive Control of Amplification).
$C+_{5a/6}$ — Add **10 µl** of **Positive Control cDNA *HCV* genotypes 5a/6 ($C+_{5a/6}$)** to the tube with **5a/6** reaction mixture (Positive Control of Amplification).

8.3.2 Amplification

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

Table 5

AmpliSens-1 amplification program for rotor-type instruments[4]

Step	Temperature, °C	Time	Fluorescent signal detection	Cycles
Hold	95	15 min	–	1
Cycling	95	5 s	–	5
	60	20 s	–	
	72	15 s	–	
Cycling 2	95	5 s	–	40
	60	20 s	FAM, JOE	
	72	15 s	–	

[1] The volumes of reagents are calculated for an amount of test samples plus 1 control of RNA extraction plus 1 extra reaction.
[2] For example, Rotor-Gene 3000 or 6000 (Corbett Research, Australia); Rotor-Gene Q (QIAGEN, Germany); CFX96 (Bio-Rad, USA); DTprime ("DNA-Technology", Russia).
[3] Number of test samples + 1 control of RNA extraction + 2 controls of PCR (N+3, N – number of test samples).
[4] For example, Rotor-Gene 3000/6000 (Corbett Research, Australia), Rotor-Gene Q (QIAGEN, Germany).

Table 6

AmpliSens-1 amplification program for plate-type instruments[5]

Step	Temperature, °C	Time	Fluorescent signal detection	Cycles
1	95	15 min	–	1
2	95	5 s	–	5
	60	20 s	–	
	72	15 s	–	
3	95	5 s	–	40
	60	30 s	FAM, JOE	
	72	15 s	–	

2. Insert the tubes into the reaction module of the device.
3. Run the amplification program with fluorescence detection.
4. Analyze results after the amplification program is completed.

9. DATA ANALYSIS

The analysis of results is performed by the software of the real-time PCR instrument used. Curves of accumulation of fluorescent signal in the channels for FAM and JOE fluorophores are analyzed.

Results are interpreted by the crossing (or not crossing) of the fluorescence curve with the threshold line set at a specific level. That determines presence (or absence) of *Ct* (cycle threshold) value of a sample in the appropriate cell of the result grid.

The result of amplification in the channel is considered *positive* if the fluorescence curve is S-shaped and crosses the threshold line in the area of reliable growth of fluorescence. The result of amplification in the channel is considered *negative* if the fluorescence curve does not have the typical shape and does not cross the threshold line (*Ct* or *Cp* value is undefined). The result of amplification in the channel is considered equivocal in all other cases.

Table 7 shows the channels for detection of *HCV* genotypes for each of the reaction mixtures applied in the PCR assay.

Table 7

Reaction mixture	1b/3a	1a/2	IC/4	5a/6
Channel for fluorophore	***HCV* genotype to be detected**			
FAM	1b	1a	IC	5a
JOE	3a	2	4	6

Interpretation of results for control samples

The result of the analysis is considered reliable only if the results obtained for the Positive and Negative Controls of amplification as well as for the Negative Control of extraction are correct (see Table 8).

Table 8

Results for controls

Reaction mixture	1b/3a		1a/2		IC/4		5a/6	
Control	Result of amplification in the channel for fluorophore							
	FAM	JOE	FAM	JOE	FAM	JOE	FAM	JOE
C–	*Ct* is absent	*Ct* is absent	*Ct* is absent	*Ct* is absent	< boundary ***Ct* value**	*Ct* is absent	*Ct* is absent	*Ct* is absent
NCA	*Ct* is absent	*Ct* is absent	*Ct* is absent	*Ct* is absent	*Ct* is absent	*Ct* is absent	*Ct* is absent	*Ct* is absent
$C+_{1b/3a}$	< boundary ***Ct* value**	< boundary ***Ct* value**	*	*	*	*	*	*
$C+_{1a/2}$	*	*	< boundary ***Ct* value**	< boundary ***Ct* value**	*	*	*	*
$C+_{IC/4}$	*	*	*	*	< boundary ***Ct* value**	< boundary ***Ct* value**	*	*
$C+_{5a/6}$	*	*	*	*	*	*	< boundary ***Ct* value**	< boundary ***Ct* value**

***** Not analyzed with the indicated reaction mixture.

NOTE: Boundary *Ct* values are specified in the *Important Product Information Bulletin* enclosed to the PCR kit.

Interpretation of results for test samples

1. The *HCV* genotype is identified by comparison of amplification results obtained with four reaction tubes (variant FRT-1g-6) in accordance with Table 7. Take into account the following:
   a) If the detected *Ct* value represents a single *HCV* genotype, then the result **"Genotype…"** is to be displayed;
   b) If two or more *Ct* values are detected for a sample, then dual, triple, etc. genotype is to be displayed. However, there is an exception:
      – If *Ct* value is detected in both the channel for JOE fluorophore for the IC/4 reaction mixture (*HCV* genotype 4) and the channel for FAM fluorophore for the 1b/3a reaction mixture (*HCV* genotype 1b) and the *Ct* value of *HCV* genotype 4 is less than the *Ct* value of *HCV* genotype 1b onto 10 cycles, then the result **"Genotype 4"** is to be displayed.
2. If only the *Ct* value of the Internal Control (IC/4 reaction mixture, channel for FAM fluorophore) is detected in the results grid and this value is less than the boundary *Ct* value specified in the *Important Product Information Bulletin*, then the result **"*HCV* genotype is not detected"** is to be displayed. Moreover, if it is known that the concentration of *HCV* RNA is within the limits of the analytical sensitivity of the PCR kit, then the result **"*HCV* genotype is not detected due to low viral load"** is to be displayed.
3. If the *Ct* values corresponding to all genotypes are absent while the *Ct* value for the Internal Control (IC/4 reaction mixture, channel for FAM fluorophore) is absent or greater than the boundary *Ct* value (specified in the *Important Product Information Bulletin*), the PCR analysis should be repeated beginning with the RNA extraction stage.

10. TROUBLESHOOTING

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

1. If the *Ct* value of at least one Positive Control of Amplification ($C+_{1b/3a}$, $C+_{1a/2}$, $C+_{IC/4}$, or $C+_{5a/6}$) is greater than the boundary *Ct* value specified in the *Important Product Information Bulletin* or absent, the PCR analysis should be repeated for all samples beginning with the RNA extraction stage.
2. If a positive signal is detected for the Negative Control of extraction (C–) with at least one of the following reaction mixtures: 1b/3a, 1a/2, 5a/6 in any channel and/or with the IC/4 reaction mixture in the channel for JOE fluorophore, PCR analysis should be repeated beginning with the RNA extraction stage for all samples that showed *HCV* genotype with this reaction mixture.
3. If a positive signal is detected for the Negative Control of amplification (NCA) in any of the channels with any reaction mixture, PCR analysis should be repeated for all samples that showed *HCV* genotype with this reaction mixture beginning with the RNA extraction stage.

11. TRANSPORTATION

AmpliSens® *HCV*-genotype-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® *HCV*-genotype-FRT** PCR kit are to be stored at temperature from minus 24 to minus 16 °C when not in use (except for Negative Control (C–), TE-buffer, and Internal Control STI-248-rec (IC)). All components of the **AmpliSens® *HCV*-genotype-FRT** PCR kit are stable until the expiry date stated on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

NOTE: Negative Control (C–), TE-buffer, Internal Control STI-248-rec (IC) are to be stored at 2–8 °C.

NOTE: PCR-mix-1-FRT *HCV* genotypes 1b/3a, PCR-mix-1-FRT *HCV* genotypes 1a/2, PCR-mix-1-FRT *HCV* IC/genotype 4, PCR-mix-1-FRT *HCV* genotypes 5a/6 are to be kept away from light.

13. SPECIFICATIONS

13.1. Analytical sensitivity

Extraction volume, µl	Nucleic acid extraction kit	PCR kit	Analytical sensitivity, IU/ml
100	**RIBO-prep**	**variant FRT-g1-6**	$5x10^3$

13.2. Analytical specificity

The analytical specificity of **AmpliSens® *HCV*-genotype-FRT** PCR kit is ensured by the selection of specific primers and probes as well as stringent reaction conditions. The primers and probes have been checked for possible homologies to all sequences published in gene banks by sequence comparison analysis.

The absence of cross-reactions between *HCV* genotypes 1a, 1b, 2, 3a, 4, 5a, and 6 was confirmed with the use of highly concentrated recombinant positive control samples and plasma samples as a part of assessment of the analytical specificity of the PCR kit.

14. REFERENCES

1. Handbook "Sampling, Transportation, and 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.
2. Guidelines to **AmpliSens® *HCV*-genotype-FRT** PCR kit for differentiation of *hepatitis C virus* (*HCV*) genotypes in the biological material by the polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection 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*-genotype-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

AmpliSens®

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

[5] For example, CFX96 (Bio-Rad, USA), DTprime ("DNA-Technology", Russia).