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For professional use only

HCV PCR detection Kit

USER MANUAL

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1. INTENDED USE

The **HCV PCR detection Kit** is intended for research and diagnostic applications. The **HCV PCR detection Kit** is an *in vitro* Nucleic Acid Test (NAT) based pathogen detection product. The **HCV PCR detection Kit** is designed to detect Hepatitis C Virus (HCV) nucleic acids in human blood plasma.

The **HCV PCR detection Kit** can be used in clinical practice for HCV diagnostics.

2. METHOD

The implemented PCR method is based on amplification of a target cDNA sequence.

The detection can be performed in each of three variants: real-time (**HCV REAL-TIME PCR Detection Kit**), endpoint (**HCV FLASH PCR Detection Kit**) and **HCV Conventional PCR Detection Kit**.

The **HCV REAL-TIME PCR Detection Kit** is based on fluorescent modification of the PCR method. The PCR-mix contains target-specific probes bearing reporter and quencher molecules. Once hybridized to a target sequence, the probes become activated. As a result of activation fluorescence increases proportionally to target sequence amplification. The intensity of fluorescence is measured at every cycle of reaction with a Real-time PCR thermal cycler data collection unit and analyzed with the software provided. The **HCV FLASH PCR Detection Kit** is based on the same principle but the fluorescence is measured only once after reaction. **HCV Conventional PCR Detection Kit** is developed for PCR result detection by electrophoresis in the agarose gel.

The automatic analysis available on “DNA-Technology” made instruments: DTlite or DTprime REAL-TIME Thermal Cyclers for HCV Real-Time PCR Detection Kit (see the catalogue at www.dna-technology.ru/en to see available supply options) and Gene or Gene-4 Fluorescence Readers REF O-GENE-EU, O-GENE4-EU for **HCV FLASH PCR Detection Kit**.

The **HCV Real-Time PCR Detection Kit** REF R3-P603-23/9EU, R3-P603-S3/9EU and R3-P603-24/9EU is also approved for use with iQ (Bio-Rad Laboratories) and Rotor-Gene Q (Qiagen) real-time thermal cyclers.

The Kit can be supplied in either separate (1x96) or stripped (8x12) tubes (REF R3-P603-23/9EU, R3-P603-24/9EU and R3-P603-S3/9EU respectively).

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-**RNA extraction**. On this step the internal control sample (RNA-IC) is added to the samples. It is needed for test quality assurance.

3. CONTENT

Table 1. *PREP-NA* DNA/RNA Extraction Kit[1]

Reagent	Description	Total volume	Amount
Lysis buffer	Light blue slightly foaming liquid	30 mL	1 vial
Precipitation buffer	Colorless liquid	40 mL	1 vial
Washout solution 1	Colorless liquid	50 mL	1 vial
Washout solution 2	Colorless liquid	30 mL	1 vial
Dilution buffer	Colorless liquid	5.0 mL (1.25 mL in each tube)	4 tubes
Negative control (C-)	Colorless liquid	3.0 mL (1.5 mL in each tube)	2 tubes
Internal control (RNA-IC)	Colorless liquid	1.0 mL	1 tube
Internal control (DNA-IC)	Colorless liquid	1.0 mL	1 tube

Table 2. Reverse RNA Transctription PCR Kit

Reagent	Description	Total volume	Amount
RT-buffer	Colorless liquid	200 µL	1 tube
RT-HAV+HCV+HDV+HGV+HIV primers and dNTPs	Colorless liquid	100 µL	1 tube
Reverse transcriptase	Colorless viscous liquid	50 µL	1 tubes

[1] - can be included into the kit if requested.

Table 3. HCV PCR detection Kit

Reagent	Description	Total volume	Amount
Paraffin-sealed PCR-mix	Colorless liquid and white waxy fractions	1.92 mL or 2.0 mL (0.02 mL per tube)	96 or 100 separate or stripped tubes of 0.2 or 0.5 mL
TECHNO Taq-polymerase	Colorless viscous liquid	50 µL	1 tube
PCR-buffer	Colorless liquid	1.0 mL (0.5 mL in each tube)	2 tubes
Positive control	Colorless liquid	150 µL	1 tube
Mineral oil (not supplied in Kit for Rotor-Gene Q)	Colorless viscous liquid	2.0 mL (1.0 mL in each tube)	2 tubes

The approximate total time needed to perform the assay is 5 hours.

Upon customer's request, optional supply of a reagent kit for DNA electrophoretic detection is possible, including:

- Electrophoresis mix (9.55 g) and Agarose gel (5 plates)

The *PREP-NA* DNA/RNA Extraction Kit is sufficient for extraction of 100 samples.

The **HCV PCR detection Kit** sufficient to test 96/100 samples including negative, positive and internal samples.

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.1 **Specimen collection**

The whole blood samples should be collected in 2.0 or 4.0 mL Vacuette type tubes with EDTA in 2.0 mg/mL final concentration. The sodium citrate anticoagulant is also applicable.


The use of heparin anticoagulant is not allowed.

4.2 **RNA extraction and PCR**

Class II biological safety cabinet;

UV PCR cabinet;

Vortex mixer;

Aspirator with trap flask to remove supernatants;

0.2, 0.5 and 1.5 mL tubes;

PCR tube rack for 0.2, 0.5 and 1.5 mL tubes;

Single channel pipettes (volume range 0.5-10 µL, 5.0-40 µL, 40-200 µL, 100-1000 µL);

RNase and DNase free filtered pipette tips (volume range 20 µL, 50 µL, 200 µL, 1000 µL);

Pipette tip without filter (for electrical laboratory aspirator);

Powder-free surgical gloves;

Disinfectant solution;

Container for used pipette tips;

High speed centrifuge (RCF 16000 x g);

Thermostat (temperature range from 40 °C to 95 °C);

Real-time PCR thermal cycler (for **HCV Real-Time PCR Detection Kit**);

Tercyc Conventional PCR Thermal Cycler (REF O-TP4-EU) or equivalent (for **HCV FLASH PCR Detection Kit and HCV Conventional PCR Detection Kit**);

Gene or Gene-4 Fluorescence Reader (REF O-GENE-EU, O-GENE4-EU) or Ala1/4 fluorescence reader or equivalent (for **HCV FLASH PCR Detection Kit**);

For electrophoretic detection:

- AC power supply;
- electrophoretic chamber;
- transilluminator;
- 1.0 L volumetric flask;
- distilled water;
- 1.0 mm diameter steel wire.

5. WARNINGS AND PRECAUTIONS

As part of industrial and personal hygiene and general safety practices, avoid all unnecessary exposure to the chemical components of this kit and ensure prompt removal from skin, eyes, and clothing upon contact.

Significant health effects are **NOT** anticipated from routine use of this kit when adhering to the instructions listed in the current manual.

5.1 General warnings and precautions

Handle and dispose all biological samples as if they were able to transmit infective agents. Avoid direct contact with the biological samples. Avoid producing spills or aerosol. Any material coming in contact with the biological samples must be treated for at least 30 minutes with disinfecting solution or autoclaved for 1 hour at 121ºC before disposal.

Handle and dispose all reagents and all materials used to carry out the assay as if they were able to transmit infective agents. Avoid direct contact with the reagents. Avoid producing spills or aerosol. Waste must be handled and disposed according to adequate safety measures. Disposable combustible material must be incinerated. Liquid waste containing acids or bases must be neutralized before disposal.

Wear suitable protective clothes and gloves and protect eyes and face.

Never pipette solutions by mouth.

Do not eat, drink, smoke or apply cosmetic products in the work areas.

Carefully wash hands after handling samples and reagents.

Dispose of leftover reagents and waste in compliance with the regulations in force.

Read the **User manual** provided with the kit before running the assay.

While running the assay follow the instructions listed in the **User manual.**

Do not use the kit after the expiry date provided.

Only use the reagents provided in the kit and those recommended by manufacturer.

Do not mix reagents from different batches.

Do not use reagents from third party manufacturers' kits.

5.2 **Warnings and precautions for molecular biology**

Molecular biology procedures, such as nucleic acids extraction, reverse transcription, amplification and detection require qualified staff to avoid the risk of erroneous results, especially due to the degradation of nucleic acids contained in the samples or sample contamination by amplification products.

Equip separate areas for the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never introduce an amplification product in the area designed for extraction/preparation of amplification reactions.

Wear lab coats, gloves and tools, which are exclusively employed for the extraction/preparation of the amplification reaction and for the amplification/detection of the amplification products. Never transfer lab coats, gloves and tools from the area designed for amplification/detection of the amplification products to the area designed for extraction/preparation of amplification reactions.

The samples must be exclusively employed for certain type of analysis. Samples must be handled under a laminar flow hood. Tubes containing different samples must never be opened at the same time. Pipettes used to handle samples must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA.

The reagents must be handled under a laminar flow hood. The reagents required for amplification must be prepared in such a way that they can be used in a single session. Pipettes used to handle reagents must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA.

Amplification products must be handled in such a way as to reduce dispersion into the environment as much as possible, in order to avoid the possibility of contamination. Pipettes used to handle amplification products must be exclusively employed for this specific purpose.

5.3 **Emergency actions**

Inhalation: Inhalation of the Master Mix contained within this kit is unlikely, however care should be taken.

Eye Contact: If any component of this kit enters the eyes, wash eyes gently under potable running water for 15 minutes or longer, making sure that the eyelids are held open. If pain or irritation occurs, obtain medical attention.

Skin Contact: If any component of this kit contacts the skin and causes discomfort, remove any contaminated clothing. Wash affected area with plenty of soap and water. If pain or irritation occurs, obtain medical attention.

Ingestion: If any component of this kit is ingested, wash mouth out with water. If irritation or discomfort occurs, obtain medical attention.

6. RNA EXTRACTION PROTOCOL

The **HCV PCR detection Kit** is designed to detect RNA extracted from blood plasma. Shake the tube containing blood sample thoroughly to mix the blood and anticoagulant.

 Using of heparin as anticoagulant is not allowed.

 The overall storage of the sample should not exceed 6 hours.

The transportation and storage temperature from collecting the sample till analysis should be from 2 °C to 8 °C.

 Whole blood cannot be frozen.

6.1 To obtain the plasma spin the tubes with blood at 800-1600 x g for 20 min at room temperature (between 18 °C and 25 °C).

 Relative centrifugal force (RCF or g) depends on rotation frequency and centrifugation radius (Annex A). To establish if your centrifuge meets the requirements apply to the exploitation manual for centrifuge.

6.2 Take the upper fraction (plasma) with an automatic sampler and put it into the new 1.5 mL tube. The blood plasma can be stored at the temperature from minus 18 °C to minus 22 °C for 3 months.

 The lysis buffer can contain the precipitate. Dissolve it at 65 °C for 10 min prior to use.

 At this step of assay use only disposable pipette tips witch have filter and are RNase and DNase free.

 If the plasma was frozen, prior to extraction thaw it at room temperature or at temperature from 2 °C to 8 °C. Vortex the thawed plasma and centrifugate at 16000 x g for 5 min. Take the volume needed for RNA extraction without touching the precepitate. Not more than 3 freezing/thawing cycles are allowed.

6.3 Mark the required number of 1.5 mL tubes by the following scheme: for each test sample and for negative control (“C-“).

For example: if you need to test 10 samples, mark 11 tubes (10 for samples, 1 for C-).

6.4 Add 10 µL of the premixed internal control (RNA-IC) in each tube.

6.5 Add 300 µL of the lysis buffer avoiding contact of the pipette tip with an edge of the tube. Close the tubes.

 Open the tube, add sample, then close the tube before proceeding to the next sample to prevent contamination.

6.6 Add 100 µL of the premixed blood plasma sample into the marked tubes. Do not add samples to the “C-“ tube.

6.7 Add 100 µL of the “C-“ into corresponding tube.

6.8 Close the tubes and mix them for 3–5 sec twice.

6.9 Incubate the tubes for 15 min at 65 °C, spin down the drops at 16000 x g for 30 sec at room temperature.

6.10 Add 400 µL of the precipitation buffer into each tube. Vortex the tubes maximally intensively for 3–5 sec twice.

6.11 Spin the tubes at 16000 x g for 15 min at room temperature.

6.12 Remove the supernatant avoiding contact of the pipette tip with the precipitate. Use new tip for each sample.

6.13 Add 500 µL of the washout solution №1 to the precipitate. Vortex the tubes for 3–5 sec. Invert the tube up and down washing of the cap of the tube. It is necessary to carry out this procedure with each tube individually.

6.14 Spin the tubes at 16000 x g for 5 min at room temperature.

6.15 Remove the supernatant avoiding contact of the pipette tip with the precipitate. Use new tip for each sample.

6.16 Add 300 µL of the washout solution №2 to the precipitate. Vortex the tubes for 3–5 sec. Gently invert the tube up and down washing of the walls and the cap of the tube. It is necessary to carry out this procedure with each tube individually.

6.17 Spin the tubes at 16000 x g for 5 min at room temperature.

6.18 Remove the supernatant avoiding contact of the pipette tip with the precipitate. Use new tip for each sample.

6.19 Open the tubes and dry the precipitate at 65 °C for 5 min strictly.

6.20 Add 16.5 µL of the dilution buffer to the precipitate. Spin down the drops for 3–5 sec.

6.21 Incubate the tubes for 10 min at 65 °C.

6.22 Spin down the drops at 16000 x g for 30 sec.

 The RNA preparation is ready. RNA should be used for reverse transcription reaction within 30 min RNA sample shouldn't be stored.

 Add 25 µL of the dilution buffer to the precipitate, if RNA sample will be used for multiple infections test.

 Increasing of dilution buffer volume will lead to a proportional RNA sample dilution and decreasing of analysis sensitivity.

7. CARRYING OUT REVERSE TRANSCRIPTION REACTION

7.1 Thaw content of "RT-Buffer" and "RT-HAV+HCV+HDV+HGV+HIV primers and dNTPs" tubes from Reverse Transcription Reagent Set at room temperature, then vortex thoroughly and spin down drops for 3-5 sec.

 RT buffer can contain the precipitate. Keep it at room temperature with intermittent vortexing until precipitate fully dissolved.

7.2 Prepare the mixture of "RT-Buffer", "RT-HAV+HCV+HDV+HGV+HIV primers and dNTPs" and reverse transcriptase (RT-mix). Add into the one plastic tube:

2.0 x (N+1) µL "RT-Buffer";
1.0 x (N+1) µL "RT-HAV+HCV+HDV+HGV+HIV primers and dNTPs";
0.5 x (N+1) µL reverse transcriptase,
N – the number of samples being analyzed, considering "C-".

 Reverse transcriptase should be kept out of freezer chamber for as short time as possible.

7.3 Vortex RT-mix and spin down drops for 3-5 sec at room temperature.

7.4 Add 3.5 µL RT-mix to each tube with isolated RNA sample and to "C-" tube. Vortex the tube and spin down the drops .

7.5 Place tubes in thermostat and incubate at 40 °C for 30 min, than incubate at 95 °C for 5 min.

7.6 Spin down condensate by centrifuging at 16000 x g for 30 sec.

cDNA preparation is ready for carrying out PCR.

 Obtained cDNA/DNA sample can be used for HAV, HDV, HGV and HIV PCR analysis. In this case PCR is performed using infection-specific reagent kit and amplification program from user manual provided with the kit. Results interpretation is performed according to instructions provided.

When it is necessary to set out PCR analysis for more than four infections:

1. Add 20 µL of the dilution buffer to the obtained cDNA/DNA sample.
2. Vortex the tubes for 3-5 sec.
3. Spin down the drops for 3-5 sec.
4. Proceed to step 8.

 Increasing of dilution buffer volume leads to proportional sample dilution and decreasing of analysis sensitivity

 The storage of the cDNA preparation is allowed at temperature from minus 18 °C to minus 22 °C for no longer than 1 month

8. PCR PROTOCOL

8.1 Mark tubes with PCR-mix for each test sample, negative control (C-), positive control (C+). Mark additionally two tubes for background buffer (applicable to FLASH PCR Detection Kits).

For example if you need to test 10 samples, mark 12 tubes (10 for samples, 1 for C-, 1 for C+). For FLASH PCR kit mark 14 tubes (10 for samples, 1 for C-, 1 for C+ and 2 for background buffer).

 Mark only the caps of the tubes when using Rotor-Gene Q Thermal Cycler.

8.2 Thaw PCR-buffer at the room temperature.

8.3 Mix the PCR-buffer and TECHNO Taq-polymerase thoroughly (3-5 sec), then spin briefly (1-3 sec) at room temperature .

 Hold TECHNO Taq-polymerase at room temperature as short time as possible. The overheating is detrimental to its performance.

8.4 Prepare the mixture of PCR-buffer and TECHNO Taq-polymerase (TECHNO Taq-polymerase solution). Add into the one tube:

10 x (N+1) µL of PCR-buffer;

0.5 × (N+1) µL of TECHNO Taq-polymerase,

N — number of the marked tubes including “C-“, “C+”, background tubes.

For example if you need to test 10 samples (12 marked tubes), prepare mixture of PCR-buffer and TECHNO Taq-polymerase for 13 (12+1) tubes: 130 µL PCR-buffer + 6.5 µL TECHNO Taq-polymerase.

8.5 Vortex the tube with TECHNO Taq-polymerase solution for 3-5 sec and spin down the drops for 1-3 sec at room temperature.

 The maximum storage time for TECHNO Taq-polymerase solution is 1 hour at temperatures between 2 °C and 8 °C.

8.6 Add 10 µL of TECHNO Taq-polymerase solution into each tube (except background tubes). Add 10 µL of background buffer into corresponding tubes (applicable to FLASH PCR Detection Kits). Avoid paraffin layer break.

8.7 Add one drop (~20 µL) of mineral oil into each tube (skip this step when using R3-P603-24/9EU). Close tubes tightly.

8.8 Vortex the tubes with samples, “C-“ and “C+” for 3-5 sec and spin down the drops for 1-3 sec at room temperature.

8.9 Add 5.0 µL of cDNA sample into corresponding tube. Avoid paraffin layer break. Do not add cDNA into the “C-“, “C+” and background (applicable to FLASH PCR Detection Kits) tubes. Avoid paraffin layer break.

 Open the tube, add cDNA/DNA sample, then close the tube before proceeding to the next cDNA/DNA sample to prevent contamination. Use filter tips.

8.10 Add 5.0 µL of "C-" which has passed NA isolation stage and reverse transcription reaction into "C-" and background (applicable to FLASH PCR Detection Kits) tubes. Add 5.0 µL of "C+" into corresponding tube. Avoid paraffin layer break.

8.11 Spin tubes briefly (1-3 sec) at room temperature (not applicable to kits approved for use with Rotor-Gene Q thermal cycler).

8.12 Set the tubes to the Thermal Cycler.

Launch the Thermal Cycler software and run PCR according to instructions supplied with device, considering 35 µL reaction mix volume. See tables 4-8 to refer the cycling program and table 9 to refer the detection channels (applicable to REAL-TIME PCR Detection Kits). Using Tercyc cycler you need to choose «Precision active regulation» regulation algorithm.

In case you use DTlite and DTprime Thermal Cyclers, launch the RealTime_PCR application in "Device operating" mode. Upload HBV_HCV_un.ini file before the first run. Add corresponding test in subsequent runs. Specify the number and identifiers of samples. Define position of tubes in software interface according to position they were set in thermal unit. Run RT-PCR.

Table 4. The PCR program for Tercyc Conventional PCR Thermal Cycler (applicable to **Conventional PCR Detection Kits and FLASH PCR Detection Kits**).

Step	For thermal cyclers with active regulation			Number of cycles
	Temperature	Time		
		min	sec	
1	94	5	0	1
2	94 62	0 0	10 20	50
3	10	…	…	Storage

 When working with FLASH PCR Detection Kits once prepared and amplified "BACKGROUND" tubes may be used many times at each PCR results detection with reaction tubes from the same lot. "BACKGROUND" tubes can be stored at the temperature from 2 °C to 8 °C and out of light for 1 month. During the detection procedure "BACKGROUND" tubes must be room temperature, for that take them out from refrigerator 1 hour before detection.

Table 5. The PCR program for DTlite and DTprime Thermal Cyclers

Step	Temperature, °C	Min	Sec	Number of cycles	Optical measurement	Type of the step
1	94.0	5	00	1		Cycle
2	94.0	0	10	50		Cycle
	62.0	0	20		v	
3	10.0	…	…	Storage		Storage

Table 6. The PCR program for iCycler iQ5 thermal cyclers (with persistent well factor)

Cycle	Repeats	Step	Dwell time	Setpoint, °C	PCR/Melt Data Acquisition
1	1				
		1	05:00	94.0	
2	50				
		1	00:10	94.0	
		2	00:20	62.0	Real Time
3		…	…	10.0	Storage

Table 7. The PCR program for iCycler iQ5 thermal cyclers (with dynamic factor)

Cycle	Repeats	Step	Dwell time	Setpoint, °C	PCR/Melt Data Acquisition
dynamicwf.tmo program					
1	1				
		1	00:30	80.0	
		2	05:00	94.0	
2	5				
		1	00:20	94.0	
		2	00:30	62.0	
3	2				
		1	00:20	80.0	Real Time
PCR program					
4	45				
		1	00:10	94.0	
		2	00:20	62.0	Real Time
5		…	…	10.0	Storage

Table 8. The PCR program for Rotor-Gene Q Thermal Cyclers

Cycling	Temperature	Hold Time	Cycle Repeats
Cycling	80 °C	300 sec	1 time
Cycling 2	94 °C 58 °C 62 °C[2]	10 sec 5 sec 25 sec	50 times

Table 9. Detection channels

	Specific product	IC
DTprime, DTlite and IQ5	Fam	Hex
Rotor-Gene Q	Green	Yellow

[2] - take the measurement

9. CONTROLS

Table 10.

Control	The controlled step	Result	Interpretation
C+	PCR	Specific signal on Fam/Green is present and the value fits in the range	Valid
		Specific signal on Fam/Green is absent	Invalid*
C-	PCR and RNA extraction	Specific signal on Fam/Green is present	Invalid**
		Specific signal on Fam/Green is absent	Valid
IC	PCR and RNA extraction	Specific signal on Hex/Yellow is present and the Cp value fits in the range	Valid
		Specific signal on Hex/Yellow is absent	Invalid***

The sample is considered positive if the signal for specific cDNA is present. The signal for IC could be absent in samples with high concentration of specific cDNA due to competitive priming.

The sample is considered negative if the signal for specific cDNA is absent and for IC is present.

* - if the signal for C+ is absent or the value does not fit in the range, all tests of current batch must be repeated.

**- If the signal for C- is present or the Cp value does not fit in the range, the result can not be reported to the patient and the test should be repeated for the sample.

10. DATA ANALYSIS

In case of using DNA-Technology made Real-Time PCR Thermal Cyclers or Fluorescence Readers the analysis performed automatically. In all other cases the analysis is based on the presence or absence of specific signal. The controls should be also considered to exclude false positive and false negative results (see p. 7 of the current manual). The cutoff Ct values for Rotor-Gene Q thermal cycler are 40 (specific product) and 33 (C+). The result characterized by Ct above this value should be considered doubtful and the whole assay should be repeated.

The interpretation should be performed in accordance with tables 11-12.

Table 11. Results with **HCV Conventional PCR Detection Kit**

Specific product (253 bp)	Internal control (480 bp)	Interpretation
Test samples		
+	Not considered	Positive
-	+	Negative
-	-	uncertain
C+		
+	Not considered	Positive
C-		
-	+	Negative

Table 12. Results with **HCV FLASH** and **REAL-TIME PCR Detection Kits**

HCV FLASH PCR Detection Kit	**Test samples**		**Interpretation**
	HCV REAL-TIME PCR Detection Kit		
	Fam/Green	**Hex/Yellow[3]**	
"+"	Cp (Ct) is specified	Not considered	Positive
"-"	Cp not specified (for iQ N/A)	Cp (Ct) 29-34	Negative
"uncertain"	Cp not specified (for iQ N/A)	Cp not specified (for iQ5 N/A)	uncertain
	C+		
"+"	Cp (Ct)<33	Not considered	Positive
	C-		
"-"	Cp not specified (for iQ N/A)	Cp (Ct) 29-34 (for Rotor-Gene Q <36)	Negative

[3] - if Cp(Ct) is more than specified, result should be considered as uncertain

11. TROUBLESHOOTING

Table 13.

	Specific signal +	Specific signal -	Possible cause	Solution
C+	-	-	Operation error PCR inhibition Violation of storage and handling requirements	Repeat whole test Dispose current batch
C-	+	+	Contamination	Dispose current batch Perform decontamination procedures
IC		-	PCR inhibition	Repeat whole test

If you face to any undescribed issues contact our representative

12. STORAGE, TRANSPORTATION AND HANDLING REQUIREMENTS

Shelf life - 12 months if all the conditions of transportation, storage and operation are met.

All components of the **HCV PCR detection Kit,** except tubes (or strips) with Paraffin sealed PCR-mix and positive control sample (C+), must be stored at the temperature from minus 18 °C to minus 22 °C during the storage period. The PCR-buffer and mineral oil can be stored at the temperature between from 2 °C to 8 °C.

 Multiple freezing-thawing of PCR buffer and mineral oil is allowed.

Tubes (or strips) with Paraffin sealed PCR-mix, positive control sample (C+) and *PREP-NA* DNA/RNA Extraction Kit must be stored at the temperature from 2 °C to 8 °C and out of light during the storage period.

Transportation of kit's components can be held by all types of roofed transport at the temperatures corresponding to the storage conditions of individual reagents, included in the kit.

An expired **HCV PCR detection Kit** must not be used.

We strongly recommend following the instructions to get robust and reliable results.

The conformity of the **HCV PCR detection Kit** to the prescribed technical requirements is subject to compliance of storage, carriage and handling conditions recommended by manufacturer.

Contact our customer service by quality issues of the **HCV PCR detection Kit**: 117587, Moscow, Varshavskoye sh. 125g building 6, floor 5, room.14, DNA Technology, LLC.

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13. SPECIFICATIONS

a. **Analytical specificity:** the **HCV PCR detection Kit** allows detection next HCV genotypes: 1a, 1b, 2a, 2b, 2c, 2i, 3, 4, 5a, 6. The samples containing HCV will be defined as positive. The samples not containing HCV will be defined as negative.

b. **Sensitivity:** not less than 200 copies of HCV RNA per 1.0 mL of blood plasma.

c. **Diagnostic sensivity:** 99.8**%**.

d. **Diagnostic specificity:** 100**%.**


The claimed specifications are guaranteed when RNA extraction is performed with *PREP-NA* DNA/RNA Extraction Kit.

14. QUALITY CONTROL

"DNA-Technology, Research&Production" LLC declares that the quality control procedures performed in accordance with ISO ISO 9001:2008 and ISO 13485:2003

15. KEY TO SYMBOLS


Caution


Manufacturer


Consult instructions for use

Negative control


Date of manufacture

Positive control


Expiration date

Cataloque number

In vitro diagnostic medical device

Sufficient for

Batch code


Temperature limitation

Version


Upper limit of temperature

Annex A
Nomogram and formula for calculation of relative centrifugal force (RCF) in the speed of rotation (RPM) depending of the rotor diameter