IVD

For professional use only

HCV PCR detection Kit

(*PREP-NA* DNA/RNA Extraction Kit included)

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: DT*lite* or DT*prime* 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 is also approved for use with iQ (Bio-Rad Laboratories) and Rotor-Gene (Qiagen) real-time thermal cyclers. The HCV FLASH PCR Detection Kit is also approved for use with Ala1/4 fluorescence reader (BioSan).

-**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

Reagent	Description	Total volume	Amount
Lysis buffer	Colorless, soapy 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
Dissolving buffer	Colorless liquid	5 ml (1.25 ml in each tube)	4 tubes
Negative control (C-)	Colorless liquid	3 ml (1.5 ml in each tube)	2 tubes
Internal control (RNA-IC)	Colorless liquid	1 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+dNTP	Colorless liquid	100 µL	1 tube
Reverse transcriptase	Colorless liquid	50 µL	1 tubes

Table 3. HCV PCR detection Kit

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Color coded composition of 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 ml (0.5 ml in each tube)	2 tubes
Positive control (C+)	Colorless liquid	150 µL	1 tube
Mineral oil (not supplied in Kit for Rotor-Gene)	Colorless viscous liquid	2 ml (1 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 or 4 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**

Vortex mixer;

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, 5-40 µL, 40-200 µL, 100-1000 µL);

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

Powder-free surgical gloves;

Disinfectant solution;

Container for used pipette tips;

High speed centrifuge (13000 rpm);

Thermostat (temperature range 50-65°C);

Physiological saline solution 0.9% NaCl (Sterile);

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

Tercyc Conventional PCR Thermal Cycler (REFO-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

The laboratory makeup should comply the requirements regulating work with microorganisms of I-IV classes of pathogenicity.

Handle and dispose all biological samples, reagents and materials used to carry out the assay as if they were able to transmit infective agents. Avoid direct contact with the biological samples reagents and materials used to carry out the assay. 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.

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.

All oligonucleotide components are produced by artificial synthesis technology according to internal quality control protocol and do not contain blood or products of blood processing.

Positive control is produced by artificial DNA synthesis technology. Positive control does not include parts of infectious agents.

All the liquid solutions are designed for single use and can not be used more than once in amplification reactions. Plastic tubes do not contain phthalates. Do not breathe gas/fumes/vapour/spray produced by the components of the kit. Do not eat/drink components of the kit. Avoid contact with eyes. 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.

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

6. RNA EXTRACTION PROTOCOL

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

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

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

6.1 To obtain the plasma spin the tubes with blood at 3000 rpm for 20 min at room temperature (18–25 °C).

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 -20°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 RNase and DNase free pipette tips.

6.3 Mark the required number of 1,5 ml tubes by the following scheme: for each test sample and for negative control (C-).

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

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

6.6 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.

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

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

6.9 Close the tubes and mix them for 3–5 s twice.

6.10 Incubate the tubes for 15 min at 65 °C, spin down the drops at 13000 rpm for 30 s at room temperature (18–25 °C).

6.11 Add 400 µL of the precipitation buffer into all tubes. Close the tubes and mix them for 3-5 s twice.

6.12 Spin the tubes at 13000 rpm for 15 min at room temperature (18–25 °C).

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

6.14 Add 500 µL of the washout solution №1 to the precipitate and shake the tube thoroughly

6.15 Spin the tubes at 13000 rpm for 5 min at room temperature (18–25 °C).

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

6.17 Add 300 µL of the washout solution №2 to the precipitate and shake the tube thoroughly.

6.18 Spin the tubes at 13000 rpm for 5 min at room temperature (18–25 °C).

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

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

6.21 Add 25 µL of the dissolving buffer to the precipitate. Spin down the drops for 3–5 s.

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

6.23 Spin down the drops at 13000 rpm for 30 s.

The RNA preparation is ready.

RNA should be use immediately for reverse transcription reaction, RNA sample shouldn't be stored!

7. CARRYING OUT REVERSE TRANSCRIPTION REACTION

7.1 Thaw content of «RT-Buffer» and «RT-HAV+HCV+HDV+HGV+HIV+dNTP» tubes from Reverse Transcription Reagent Set at room temperature, then vortex thoroughly and spin down drops by centrifuging at 1000-3000 RPM for 3-5 sec.

7.2 Prepare the mixture of RT Buffer, «RT-HAV+HCV+HDV+ HGV+HIV+dNTP» and reverse trancriptase (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+dNTP»,
0,5 x (N+1) µl reverse transcriptase,
where N+1 – the number of samples being analyzed, considering «C-» (N) and one extra sample .


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

7.3 Vortex RT-mix obtained and spin down drops by centrifuging at 1000-3000 RPM for 3-5 sec.

7.4 Add 3,5 µl RT-mix to each tube with isolated RNA sample and to «C-» tube.

7.5 Vortex tubes and sediment drops with centrifuging at 1000-3000 RPM for 3-5 sec.

7.6 Place tubes in thermostat and incubate at 40oC for 30 min, than incubate at 95oC for 5 min.

7.7 Spin down condensate by centrifuging at 13000 RPM for 30 sec.

cDNA preparation is ready for carrying out PCR.

Note. cDNA storage at -20^{o}C for not longer than one month is tolerated.

The nucleic acid preparation is ready.

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 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 each sample, 1 for C-, 1 for C+ and 2 for background buffer).


Mark only the caps of the tubes when using Rotor-Gene 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 (18–25 °C).

 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 seconds and spin down the drops for 1-3 seconds at room temperature (18–25 °C). The maximum storage time for Taq-polymerase solution is 1 hour.

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 kits). Avoid paraffin layer break.

8.7 Add one drop (~20 µL) of mineral oil into each tube (not applicable to kits approved for use with Rotor-Gene thermal cycler). Close tubes tightly.

8.8 Vortex the tubes with samples for 3-5 seconds and spin down the drops for 1-3 seconds.

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 kits) tubes. Avoid paraffin layer break.

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

8.10 Add 5 µL of C- which passed whole RNA extraction procedure and reverse transcription into C- and background (applicable to FLASH PCR kits) tubes. Add C+ into corresponding tube. Avoid paraffin layer break.

8.11 Spin tubes briefly (1-3 sec).

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 4-9 to refer the cycling program and table 10 to refer the detection channels (applicable to Real-Time PCR kits). Using Thercyc cycler you need to choose «Precision active regulation» regulation algorithm.

Table 4. The PCR program for Tercyc Conventional PCR Thermal Cycler (applicable to **Conventional PCR kits**).

Step	For thermal cyclers with active regulation			Number of cycles
	Temperature	Time		
		min	sec	
1	94	5	0	1
2	94 64 72	0 0 0	20 5 5	5
3	94 64 72	0 0 0	5 5 5	40
4	10	…	…	Storage

Table 5. The PCR program for Tercyc Conventional PCR Thermal Cycler (applicable to **FLASH PCR kits**).

Step	For thermal cyclers with active regulation			Number of cycles
	Temperature	Time		
		min	sec	
1	94	5	0	1
2	94 64 67	0 0 0	5 5 5	5
3	94 64 67	0 0 0	1 5 5	40
4	10	…	…	Storage

Table 6. The PCR program for DT*lite* and DT*prime* Thermal Cyclers

Step	Temperature, °C	Min.	Sec.	Number of cycles	Optical measurement	Type of the step
1	94	5	00	1		Cycle
2	94	0	10	50		Cycle
	62	0	20		v	
5	10	…	…	Storage		Storage

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

Cycle	Repeats	Step	Dwell time	Setpoint, ºC	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 8. The PCR program for iCycler iQ5 thermal cyclers (with dynamic factor)

Cycle	Repeats	Step	Dwell time	Setpoint, ºC	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 9. The PCR program for Rotor-Gene Thermal Cyclers

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

* Take the measurement

Table 10. Detection channels

	Specific product	IC
DT*prime*, DT*lite* and IQ5	FAM	HEX
Rotor-Gene	Green	Yellow

9. CONTROLS

Table 11.

Control	The controlled step	Result		Interpretation
		Specific signal is present	Specific signal is absent	
C+	PCR	+	+	Valid
		-	-	Invalid
C-	PCR and RNA extraction	+	+	Invalid
		-	-	Valid
IC	PCR and RNA extraction	+	+	Valid
		-		Valid
			-	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 present, whole tests of current batch considered false. Decontamination required.

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 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 12-13.

Table 12. 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 13. 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**	
"+"	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 is specified	Not considered	Positive
	C-		
"-"	Cp not specified (for iQ N/A)	Cp (Ct) 29-34	Negative

11. TROUBLESHOOTING

Table 14.

	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 AND HANDLING REQUIREMENTS

Expiry date – 9 month from the date of production.

All components of the **HCV PCR detection Kit** (except PCR-mix and C+) must be stored at -20 °C over the storage period. The PCR-buffer and mineral oil can be stored at at 2-8 °C.

The PCR-mix, C+ and *PREP-NA* DNA/RNA Extraction Kit must be stored at 2-8 °C over the storage period.

Transportation can be held by all types of roofed transport with adherence to above mentioned temperature requirements.

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**: 115587, Moscow, Varshavskoye sh. 125g building 6, DNA Technology, LLC. Phone/Fax: +7(495)9804555 e-mail: help@dna-technology.ru, www.dna-technology.ru

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 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