Customer service department:
Phone:+7(495)640.16.93,
Phone/Fax: +7(495)640.17.71.
E-mail: hotline@dna-technology.ru,
www.dna-technology.ru

318-2 2021-07-21

HCV Real-time PCR genotyping Kit

General information

Intended use:
HCV Real-time PCR genotyping Kit is intended for detection of Hepatitis C virus and its genotyping in samples of peripheral blood plasma by real-time PCR method.
The kit can be used for *in vitro* diagnostics of 1a, 1b, 2 and 3a/3b.

Method:
Reverse transcription followed by quantilitative PCR.

Samples:
Peripheral blood plasma.

RNA extraction:
The DNA-Technology PREP-NA DNA/RNA extraction kit.

Features
Internal control sample (RNA–IC) is included in PREP-NA DNA/RNA extraction kit content and added to a sample being tested at the stage of nucleic acids extraction. That allows evaluation of all testing stages.
Positive control plasmid ("C+") supplied with the kit is intended for specific PCR assessment.
We also recommend including in assay the negative control ("C-") which is not supplied but very helpful for contamination control purposes. Use deionized water or sterile buffered saline instead of sample, starting from extraction step.

Devices:
The automatic analysis for HCV Real-time PCR genotyping Kit (i.e. genotype calls) is available on "DNA-Technology" made DTlite[1] and DTprime[2] REAL-TIME Thermal Cyclers; software version is not lower than 7.3; the current version of the software is available for download at http://www.dna-technology.ru/eng/support/.
The HCV Real-time PCR genotyping Kit is also approved for use with iQ5 (Bio-Rad Laboratories) real-time thermal cyclers.

⚠ Please enquire company's representative about compatibility of third-party Real-time instruments.

Overall time needed to perform the analysis (including sample preparation procedure):
5 hours.

Number of tests:
48

Kit contents:

Reagent	Quantity	
Kit for RNA extraction		
Lysis buffer	15 mL	1 vial
Precipitation buffer	20 mL	1 vial
Washout solution №1	25 mL	1 vial
Washout solution №2	15 mL	1 vial
Dilution buffer	1.25 mL	2 tubes
Negative control ("C-")	1.5 mL	1 tube
Internal control (RNA-IC)	500 µL	1 tube
Kit for reverse transcription		
RT-buffer	200 µL	1 tube
RT-HCV-typing primers +dNTP's ("RT-primers+dNTP's")	50 µL	1 tube
Reverse transcriptase	25 µL	1 tube
PCR detection Kit		
Paraffin sealed PCR-mix	20 µL	48 tubes for each genotype (1a, 1b, 2, 3a/3b) and 48 tubes for HCV-common
TECHNO Taq-polymerase	150 µL	1 tube
PCR-buffer	1.0 mL	3 tubes
Buffer for cDNA dilution	1.25 mL	1 tube
Mineral oil	1.0 mL	6 tubes
Positive control ("C+")	75 µL	1 tube for each genotype (1a, 1b, 2, 3a/3b) and 1 tube for HCV-common

[1] - supported by 4S1, 4S2, 5S1, 5S2, 6S1, 6S2 instruments
[2] - supported by 4M1, 4M3, 4M6, 5M1, 5M3, 5M6, 6M1, 6M3, 6M6 instruments

Dye label detection channels

Fam	Hex	Rox	Cy5	Cy 5.5
HCV	RNA-IC	-	-	-

Procedure

1 RNA-extraction

The HCV Real-time PCR genotyping Kit is designed to detect RNA extracted from human blood plasma samples. The HCV Real-time PCR genotyping Kit has passed clinical and analytic validation with PREP-NA DNA/RNA extraction kit. Other RNA extraction kits providing pure RNA sample sufficient by RNA copy quantity are also allowed.

1.1 Mark the required number of the 1.5 mL tubes: one tube for each sample and negative control "C-".

1.2 Perform the extraction procedure according to user manual supplied with the RNA extraction kit.

The overall storage of the sample should not exceed 6 hours. The transportation and storage temperature from collecting the sample till analysis should be between 2 °C and 8 °C range.

The lysis buffer supplied with PREP-NA DNA/RNA extraction kit 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.

2 Preparing reverse transcription

2.1 Thaw content of RT-buffer and "RT-primers+dNTP's" tubes from Reverse Transcription Kit at room temperature, then vortex thoroughly and spin down drops by centrifuging at 1000-3000 RPM for 3-5 sec.

The RT-buffer can contain the precipitate. Dissolve it at temperatures between 18 °C and 25 °C prior to use.

2.2 Prepare RT-mix. Mix in the separate tube:
2.0 x (N+1) µL of RT-buffer,
1.0 x (N+1) µL of "RT-primers+dNTP's",
0.5 x (N+1) µL of reverse transcriptase,
N+1 – is a quantity of the samples to be tested taking to account "C-" (N) and one extra sample.
Example: for testing of 5 samples, marked tubes - 6; mix 14 µL of RT-buffer, 7 µL of primers and 3.5 µL of reverse transcriptase (calculate final volume for 7 (6+1) tubes).

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

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

2.4 Add 3.5 µL of the RT-mix to each tube with 16.5 µL of isolated RNA sample and to "C-" tube. Pipette 5-7 times to mix the content of the tube.

2.5 Place tubes in thermostat and incubate at 40 °C for 30 min, then heat up to 95 °C and leave for 5 min.

Use "DNA-Technology" Gnom Programable thermostat or similar thermostats with clamping cover.

2.6 Spin the tubes at 13000 RPM for 30 sec to collect the drops.

2.7 Add 10 µL of the cDNA dilution buffer to the obtained preparation of cDNA.

2.8 Vortex and spin the tubes at 1000-3000 RPM for 3-5 sec to collect the drops.

The cDNA preparation is ready for PCR.

You can also use cDNA obtained with HCV REAL-TIME PCR detection Kit and HCV Quantitative REAL-TIME PCR Kit. In this case before the PCR follow the steps listed in 2.7–2.8.

Add the sample and corresponding "C+" to HCV-common tube for control of presence/absence of other Hepatitis C virus (HCV) genotypes in peripheral blood plasma sample. If the patient has HCV infection but the viral genotype differs from those detected with HCV Real-time PCR genotyping Kit the HCV-common will give positive result thus confirming the infection by other HCV genotype. HCV-common kit shouldn't be used when testing cDNA obtained with an aid of HCV REAL-TIME PCR detection Kit and HCV Quantitative REAL-TIME PCR Kit.

3 PCR amplification

3.1 Mark the required number of the tubes with paraffin sealed PCR-mix from each kit (1a type, 1b type, 2 type, 3a/3b type and HCV-common): for the each sample to be tested, for negative control and for positive control.

Example: for simultaneous testing of 5 samples per 4 HCV genotypes in one PCR run, mark 25 tubes for samples, 5 tube for "C-" and 5 tubes for "C+". The resulting number of tubes is 35.

3.2 Vortex the PCR-buffer and TECHNO Taq-polymerase thoroughly (3-5 sec), then spin briefly (1-3 sec) at room temperature (between 18 °C and 25 °C).

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

3.3 Prepare Taq-polymerase solution. Mix in the separate 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+”.

Example: for testing of 5 samples, marked tubes - 35, prepare mixture of PCR-buffer and TECHNO Taq-polymerase for 36 (35+1) tubes: 360 µL PCR-buffer + 18 µL TECHNO Taq-polymerase.

3.4 Add 10 µL of TECHNO Taq-polymerase and PCR buffer mixture into each tube. Avoid paraffin layer break.
3.5 Add one drop (~20 µL) of mineral oil into each tube. Close tubes tightly.
3.6 Add 5.0 µL of the premixed cDNA sample into corresponding PCR-tubes. Open the tube, add cDNA sample, then close the tube before proceeding to the next cDNA sample to prevent contamination. Use filter tips. Do not add cDNA into the “C-”, “C+” tubes.
3.7 Add 5.0 µL of the premixed “C-” which passed whole RNA extraction procedure and reverse transcription into “C-” tube. Add 5.0 µl of the premixed “C+” into corresponding tube. Avoid paraffin layer break.
3.8 Spin tubes briefly at 1000 RPM for 3-5 sec.
3.9 Set the tubes to the Thermal Cycler.

For DTlite and DTprime thermal cyclers:
Launch the RealTime_PCR application in “Device handling” mode. Upload ini file “HCVtyp.ini” before the first run. Add test “HCVtyp” in subsequent runs. Specify the number and identificator of samples. Define position of tubes in software interface according to position they were set (3.9) in thermal unit. Run PCR.
For iQ5 thermal cyclers:
Turn on the device and the power supply of the device’s optical part, leave to heat for 30 minutes. Run Software iCycler (or Bio-Rad iQ5). Create and save a new protocol when the given type of the test for the first time. In subsequent productions select the saved protocol, install configuration of the plate (file with data of the sample ID’s and their position in the plate) and run PCR according the volume of reaction mix (35 µL) (Tables 1 and 2).

Table 1.

The PCR program for iCycler iQ

Cycle	Repeats	Step	Dwell time	Setpoint, ºC	PCR/Melt Data Acquisition
Well dynamic factor readout program(dynamicwf.tmo)					
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 2.

The PCR program for 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

4 **The PCR and post-PCR analysis** are operated by software and held in automatic mode.

Table 3.

PCR results interpretation

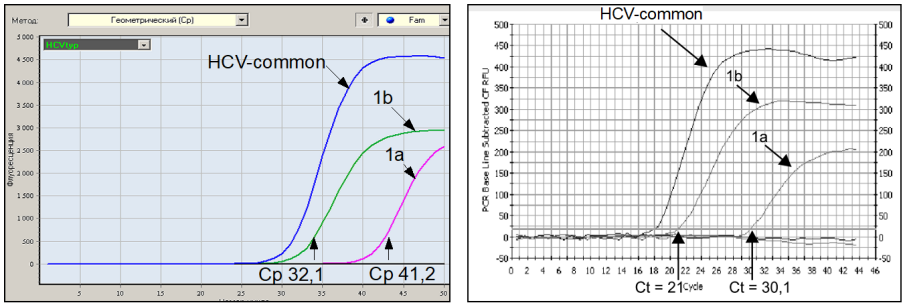
FAM detection channel		**HEX detection channel**	**Interpretation**
One of the genotypes HCV (1a, 1b, 2, 3a/3b)	**HCV-common**	**Internal control**	
Analyzed samples			
Cp(Ct)<40*	Cp(Ct)<40*	Be ignored	RNA of HCV genotype ____ is present
Cp(Ct) not reported	Cp(Ct) not reported	Cp(Ct)≤36*	RNA of HCV is absent
Cp(Ct) not reported	Cp(Ct)<40*	Be ignored	RNA of undefined genotype HCV is present **
Cp(Ct) not reported	Cp(Ct) not reported	Cp(Ct) not reported or Cp(Ct)>36*	Unreliable result
Positive control "C+"* **			
Cp(Ct)≤33	Cp(Ct)≤33	Be ignored	Positive result
Negative control "C-" * **			
Cp(Ct) not reported	Cp(Ct) not reported	Cp(Ct)≤36	Negative result

* - If the Cp(Ct) value on the FAM channel exceed 40 or is not reported and Cp(Ct) value on the HEX channel exceed 36 repeating of analysis starting from RNA extraction is recommended.

** - Hepatitis C virus is characterized by high polymorphism which causes great variety of genotypes and subtypes. Therefore, a positive result on in HCV-common and the lack of signal on all 4 genotype, proposed in this kit, can indicate the presence of other HCV variants in the test sample.

*** - If the Cp(Ct) values exceed the specified values the result is unreliable.

When obtaining two genotypes for one sample (for example genotypes 1a и 1b) with the Cp/Ct interval more than 3 cycles, the less Cp/Ct value curve should be considered positive. The more Cp/Ct value curve should be considered unspecific cross-reaction (fig.1).



A) B)

Fig 1. Interpretation: HCV of the genotype 1b is present.

A) – for DTlite and DTprime B) – for iCycler iQ5

Storage and handling requirements

The PCR and reverse transcription chemistry, except paraffin sealed PCR-Mix, should be stored at – 20 °C through the storage period.

The tubes with paraffin sealed PCR-Mix, should be stored in a dark place at temperatures between 2 °C and 8 °C through the storage period.

Shelf-life – 9 months since the date of production.

Contact our customer service department regarding quality issues with the Hepatitis C virus Real-time PCR genotyping Kit:
117587, Russia, Moscow, int. ter. Municipal District Chertanovo Severnoye, Varshavskoye shosse, 125 Zh, building 5, floor 1, office 12, "DNA-Technology" LLC
8 800 200-75-15 (toll-free call for Russia)
+7 (495) 640-16-93 (chargeable call for CIS and foreign countries)
e-mail: help@dna-technology.ru
www.dna-technology.ru

Number: 318-2
2021-07-21