# HCV Real-time PCR genotyping Kit

## General information

### Overview:
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 most genotypes of HCV widely distributed in Russia (1a, 1b, 2 and 3a/3b).

### Method:
Reverse transcription followed by qualitative PCR.

### Samples:
Peripheral blood plasma.

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

### Controls:
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.

### Devices:
The automatic analysis for HCV Real-time PCR genotyping Kit (i.e. genotype calls) is available on “DNA-Technology” made DT-lite and DTprime REAL-TIME Thermal Cyclers. The HCV Real-time PCR genotyping Kit is also approved for use with iQ5 (Bio-Rad Laboratories) real-time thermal cyclers.

### Important!
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.

### The number of tests:
48

## Content:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Kit for RNA extraction</strong></td>
<td></td>
</tr>
<tr>
<td>Lysis buffer</td>
<td>15 mL</td>
</tr>
<tr>
<td>Precipitation buffer</td>
<td>20 mL</td>
</tr>
<tr>
<td>Washout solution №1</td>
<td>25 mL</td>
</tr>
<tr>
<td>Washout solution №2</td>
<td>15 mL</td>
</tr>
<tr>
<td>Dissolving buffer</td>
<td>1.25 mL</td>
</tr>
<tr>
<td>Negative control (&quot;C-&quot;)</td>
<td>1.5 mL</td>
</tr>
<tr>
<td>Internal control (RNA-IC)</td>
<td>500 μL</td>
</tr>
<tr>
<td><strong>Kit for reverse transcription</strong></td>
<td></td>
</tr>
<tr>
<td>RT-buffer</td>
<td>100 μL</td>
</tr>
<tr>
<td>RT-HCV-typing+dNTP’s (&quot;RT-primers+dNTP’s&quot;)</td>
<td>50 μL</td>
</tr>
<tr>
<td>Reverse transcriptase</td>
<td>25 μL</td>
</tr>
<tr>
<td><strong>PCR detection Kit</strong></td>
<td></td>
</tr>
<tr>
<td>Paraffin sealed PCR-mix</td>
<td>20 μL</td>
</tr>
<tr>
<td>TECHNO Taq-polymerase</td>
<td>150 μL</td>
</tr>
<tr>
<td>PCR-buffer</td>
<td>1.0 mL</td>
</tr>
<tr>
<td>Dissolving buffer</td>
<td>1.25 mL</td>
</tr>
<tr>
<td>Mineral oil</td>
<td>1.0 mL</td>
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<tr>
<td>Positive control (&quot;C+&quot;)</td>
<td>75 μL</td>
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</table>

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

<table>
<thead>
<tr>
<th>Fam</th>
<th>Hex</th>
<th>Rox</th>
<th>Cy5</th>
<th>Cy 5.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCV</td>
<td>RNA-IC</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Procedure overview

1. **RNA-extraction**

   The HCV Real-time PCR genotyping Kit is designed to detect RNA extracted from human blood plasma samples. Perform the extraction procedure according to user manual supplied with the RNA extraction kit. 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.

   - 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.
   - 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 supplied with PREP-NA DNA/RNA extraction kit can contain the precipitate. Dissolve it at 18-25 °C prior to use.

   2.2. Prepare RT-mix. Add to the one 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 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 dissolving buffer from PREP-NA DNA/RNA Extraction Kit 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 p. 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. **Preparing PCR**

   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
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.

### 3.3
Prepare Taq-polymerase solution. Add into the one tube:
- 10 x (N+1) μL of PCR-buffer,
- 0.5 x (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 Taq-polymerase solution 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 (p. 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).

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<table>
<thead>
<tr>
<th>Cycle</th>
<th>Repeats</th>
<th>Step</th>
<th>Dwell time</th>
<th>Setpoint, °C</th>
<th>PCR/Melt Data Acquisition</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>00:30</td>
<td>80.0</td>
<td></td>
</tr>
<tr>
<td>2</td>
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<td>5</td>
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<tr>
<td>3</td>
<td>2</td>
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<td>45</td>
<td>1</td>
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<td></td>
<td></td>
<td></td>
<td>10.0</td>
<td>Storage</td>
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</table>

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* - 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.

Table 1. The PCR program for iCycler iQ5 thermal cyclers (with dynamic factor)
4. **The PCR and post-PCR analysis** are operated by software and held in automatic mode.

**Table 3. Interpretation of PCR**

<table>
<thead>
<tr>
<th>Exponential fluorescence curve (positive result)</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>One of the genotypes HCV (1a, 1b, 2, 3a/3b)</td>
<td>HCV–common</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
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<td>-</td>
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<tr>
<td>-</td>
<td>-</td>
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<tr>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

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).

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

A – for DTlite and DTprime  
B – for iCycler iQ5

Please be aware that 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.

**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 2–8 °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:  
115587, Moscow, Varshavskoye sh. 125g building 6, DNA Technology  
Phone/Fax: +7(495)9804555  
e-mail: help@dna-technology.ru  
www.dna-technology.ru

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