

**Instructions for Use****RealLine HCV Genotype Qualitative  
Uni-Format**










**QUALITATIVE ASSAY KIT FOR THE DETECTION OF RNA AND DIFFERENTIATION OF  
GENOTYPES 1, 2, 3 OF HEPATITIS C VIRUS BY REAL TIME PCR**

Research Use Only (RUO)

<b>RealLine HCV Genotype Qualitative Uni-format</b>	<b>VBD0793</b>	<b>48 Tests</b>
<b>valid from:</b>	<b>November 2019</b>	

## RealLine HCV Genotype Qualitative Uni-Format

### Explanation of symbols used in labeling

	For research use only
	Batch code
	Catalogue number
	Contains sufficient for <n> tests
	Use-by-date
	Temperature limit
	Consult instructions for use
	Manufacturer
	Keep away from sunlight



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Rotor-Gene® is a registered trademark of Qiagen Group, Germany.

## RealLine HCV Genotype Qualitative Uni-Format

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## RealLine HCV Genotype Qualitative Uni-Format

### QUALITATIVE ASSAY KIT FOR THE DETECTION OF RNA AND DIFFERENTIATION OF GENOTYPES 1, 2, 3 OF HEPATITIS C VIRUS BY REAL TIME PCR

Research Use Only

#### 1. INTENDED USE

The assay kit RealLine HCV Genotype Qualitative (Uni-format) is intended for the detection of Hepatitis C virus RNA and differentiation of genotypes 1, 2, 3. Samples to use are blood plasma or serum.

#### Overview to the detected Genotypes:

Channel	HEX JOE Yellow	FAM Green	ROX Orange	Cy5 Red
HCV Genotypes	1a; 1b;	2a, 2b; 2c; 2i;	3a; 3b;	1a; 1b; 2a; 2b; 2c; 2i; 3a; 3b; 4; 5a; 6

The assay kit RealLine HCV Genotype Qualitative (Uni-format) contains reagents sufficient for 48 test runs, including control samples. The extraction of RNA from samples is performed using the RealLine Extraction 100 or the RealLine Extraction 1000 kits.

The kit is intended for use with block-type cyclers RealLine Cyler (BIORON Diagnostics GmbH), iQ™ iCycler, iQ5™ iCycler, CFX96™ (Bio-Rad, USA), DT96 (DNA-Technology, Russia) and rotor type cyclers Rotor-Gene® 3000, 6000 and Rotor-Gene® Q (Qiagen, Germany).

The use of other real-time PCR devices and of appropriate reaction volumes has to be validated in the lab by the user.

#### 2. KIT CONTENTS

<b>Complex Positive Control sample (PC)</b> , lyophilized	1 vial
<b>Ready Master Mix</b> for reverse transcription and PCR, lyophilized, for HCV RNA 1/2/3 RNA (RMM) determination	48 test tubes
<b>Recovery Solution for Controls (RSC)</b>	1 vial, 4 ml

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### 3. PRINCIPLES OF THE PROCEDURE

The method of analysis is based on conducting the reverse transcription of selected fragment of RNA and subsequent amplification of cDNA and detection of PCR products in real time.

Analysis of each specimen is carried out in a separate tube, which contains the lyophilized Ready Master Mix for RT-PCR (RMM).

Determination of genotype of HCV RNA is provided by independent detection of fragments of HCV genome, corresponding to different genotypes, due to fluorogenic gene-specific probes, detected through different channels (see. Table). To confirm the presence of HCV RNA in the specimen in sufficient for genotyping quantities, the amplification of HCV cDNA is additionally recorded in the same test tube due to cleavage of fluorogenic probe, corresponding to any HCV variant (irrespective of genotype), and recorded through Cy5 channel.

To control the RMM, each run uses Complex Positive Control sample (PC) containing HCV RNA of genotypes 1b, 2a, 3a.

### 4. SPECIFICATIONS

#### 4.1. Sensitivity

The kit allows determining the HCV genotype within the RNA concentration range not less than 400 IU/ml with RNA extraction from 1.0 ml of the specimen.

#### 4.2. Specificity

The kit detects the Hepatitis C virus of genotypes 1a, 1b, 2a, 2b, 2c, 2i, 3, 4, 5a, and 6, irrespective of the subtype, and differentiates genotypes 1 (subtypes 1a, 1b), 2 (subtypes 2a, 2b, 2c, 2i), and 3 (subtypes 3a, 3b).

In samples that do not contain HCV RNA, the analysis result should be negative in 100 % of samples.

### 5. PRODUCT USE LIMITATIONS

For Research Use Only.

Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

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### 6. WARNING AND PRECAUTIONS

- ☞ For in vitro use only.
- ☞ The kits must be used by skilled personnel only.
- ☞ When handling the kit, follow the national safety requirements for working with pathogens.
- ☞ To prevent contamination, the stages of RNA isolation and PCR test run must be spatially separated.
- ☞ Avoid microbial and nuclease contamination of reagents when removing aliquots from reagent vials.
- ☞ Wear protective disposable gloves, laboratory coats and eye protection when handling specimens and kit reagents.
- ☞ Every workplace must be provided with its own set of variable-volume pipettes, necessary auxiliary materials and equipment. It is prohibited to relocate them to other workplaces.
- ☞ The use of sterile disposable pipette tips is recommended.
- ☞ Never use the same tips for different samples.
- ☞ Once the work is completed, expose all working surfaces and equipment to UV bactericidal lamps for 1 hour to disinfect and prevent contamination. Then, treat them with disinfecting agents prescribed by the local sanitary rules.
- ☞ Do not pool reagents from different lots or from different vials of the same lot.
- ☞ Dispose unused reagents and waste in accordance with country, federal, state and local regulations.
- ☞ Do not use the kit after the expiration date at the side label of the box.

### 7. EQUIPMENT AND MATERIALS REQUIRED BUT NOT SUPPLIED

- **RNA extraction kit:** RealLine Extraction 100 or RealLine Extraction 1000;
- Real-time PCR cycler;
- Laminar safety box;
- Refrigerator;
- Microcentrifuge;
- Half-automatic variable-volume single-channel pipettes;
- Disposable medical non-sterile powder-free gloves;
- Disposable pipette tips with aerosol filter;
- Racks for 0.2 ml tubes;
- Biohazard waste container.

If the kit is used with an extraction kit from another supplier:  
Negative Control Sample (negative tested Plasma or Serum)

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### 8. PREPARATION OF SPECIMENS

The assay is performed on extracted RNA samples obtained from the material, PC and NC using one of the NA extraction kits listed in chapter 1, according to the Instruction Manual to the kit.

Each group of samples undergoing the procedure of RNA extraction should include a PC from this kit and a Negative Control sample (NC) which is a component of the NA extraction kit.

When using a kit of another supplier for the extraction of nucleic acids as recommended in chapter 1, each tube.

- For the **NC** use 100 µl of the Negative Control Sample.
- For the **PC** use 70 µl of Negative Control Sample and 30 µl of Positive Control to the tube marked PC.

### 9. PROCEDURE

#### 9.1 Preparation of kit components

Prior to the preparation of the specimens, add **1 ml of RSC** to the tube with PC, tightly close the tube with a new plastic cap provided with the kit. Carefully mix, keep at room temperature for 15 min and then thoroughly mix again.

*After initial opening of the tube, store PC at (2 – 8) °C for no more than 1 month.*

Prior to the test, take the kit out of the refrigerator and keep the **Ready Master Mix (RMM)** closed in the package at (18 – 25) °C for at least 30 min. Then open the package and take off the necessary number of tubes with RMM (including the specimens and control samples). Put the remaining tubes immediately back into the foil pouch, squeeze the air out and tightly close it with a clip.

*After initial opening, store RMM at (2 – 8) °C for no more than 3 months.*

**9.2** Place the tubes with the prepared specimens in the magnetic rack.

**9.3** Label the tubes with RMM for each specimen and control sample and place in the rack.

**9.4** Add **50 µl** of the corresponding extracted RNA solution to each tube using a separate pipette tip with filter. Close the tubes tightly.

**9.5** Place the tubes into the real-time PCR cycler.

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9.6 Program the real-time PCR cycler as follows:

### For Rotor-Gene 3000/6000/Q

Step 1:	45 °C	30 min	
Step 2:	94 °C	1 min	
Step 3:	94 °C	10 sec	50 Cycles
	60 °C*	<b>40 sec</b>	
* measurement of fluorescence at 60 °C			

### For RealLine Cyclers, iQ/iQ5 iCycler, CFX96, DT-96

Step 1:	45 °C	30 min	
Step 2:	94 °C	1 min	
Step 3:	94 °C	10 sec	50 Cycles
	60 °C*	<b>20 sec</b>	
* measurement of fluorescence at 60 °C			

9.7 In accordance with the Table, set the channels for fluorescence measurement depending on the cycler in use

	<b>RealLine Cycler, iQCycler, CFX96, DT-96</b>	<b>Rotor-Gene 3000 (6000, Q)</b>
<b>Genotype 1</b>	HEX	JOE ( <i>Yellow</i> )
<b>Genotype 2</b>	FAM	FAM ( <i>Green</i> )
<b>Genotype 3</b>	ROX	ROX ( <i>Orange</i> )
<b>All genotypes</b>	Cy5	Cy5 ( <i>Red</i> )

9.8 Program the positions of test tubes with samples, positive and negative controls according to the instruction manual for the real time PCR system in use.

9.9 Run the program.



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### 10. DATA ANALYSIS AND INTERPRETATION

- 10.1** For **PC** the program should detect an increase in HCV cDNA amplification signal through all channels: **HEX** (HCV genotype 1)  
**FAM** (HCV genotype 2)  
**ROX** (HCV genotype 3)  
**Cy5** (all HCV genotypes)  
and determine the threshold cycle, **PC Ct**;
- 10.2** The results of an individual PCR run are to be analysed and evaluated if **PC Ct** through **FAM**, **HEX**, **ROX** and **Cy5** channels is within the range indicated in the insert for the kits of this lot (label on the inside of the lid of the box).
- 10.3** For **NC**: **No FAM**, **HEX**, **ROX**, **Cy5** fluorescence increase should appear (no HCV cDNA amplification).
- 10.4** If the Ct value for **NC** through the **FAM**, **HEX**, **ROX** or **Cy5** channel is **less than or equal to 40**, it indicates the presence of contamination.

In case of contamination all positive results of this individual RT-PCR test run are considered as **equivocal**. Actions are required to identify and eliminate the source of contamination, and repeat the analysis of all samples of this run that were identified positive. Samples that showed negative results in this run should be considered as negative.

#### Determination of the HCV genotype:

- 10.5** The specimen contains **HCV genotype 1** if the **Ct** through **HEX** and **Cy5** channels **are less than or equal to 40**.
- 10.6** The specimen contains **HCV genotype 2** if the **Ct** through **FAM** and **Cy5** channels **are less than or equal to 40**.
- 10.7** The specimen contains **HCV genotype 3** if the **Ct** through **ROX** and **Cy5** channels **are less than or equal to 40**.
- 10.8** The specimen contains HCV RNA with a genotype different from 1 (*1a*, *1b*), 2 (*2a*, *2b*, *2c*, *2j*), and 3 (*3a*, *3b*) if the positive amplification result is observed through **Cy5** channel, and the **Ct** value through **FAM**, **ROX**, or **HEX** channels **less than or equal to 40** is not detected.
- 10.9** If the concentration of HCV RNA in the specimen is below 400 IU/ml, the sensitivity of the kit could be insufficient to determine the HCV genotype. The RNA concentration could be determined using the assay kits **RealLine HCV quantitative (Str-format) VBD0794** and **RealLine HCV quantitative (Uni-Format) VBD0799**.

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Genotype	HEX JOE Yellow	FAM Green	ROX Orange	Cy5 Red
1 (1a; 1b)	+		-	+
2 (2a; 2b; 2c; 2i)		+	-	+
3 (3a; 3b)	-	-	+	+
4; 5a; 6	-	-	-	+
Negative	-	-	-	-
Positive Control	+	+	+	+

### 11. STORAGE AND TRANSPORTATION

- Store the assay kit at (2 – 8) °C in the manufacturer’s packing.
- Transport at (2 – 8) °C. Transportation at up to 25 °C for up to 10 days is allowed.
- Do not freeze the kit!
- Strictly follow the Instruction manual for reliable results.
- Do not use kits with damaged inner packages and get in contact with BIORON Diagnostics GmbH.
- Storage and shelf life of solutions and components of the kit after initial opening:
  - Positive Control sample: 1 month at (2 – 8) °C after dilution.
  - Ready Master Mix (MM) in Uni-format: at (2 – 8) °C for up to 3 month.

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### **ANNEX I: SETTINGS FOR REALLINE CYCLER AND DT96:**

for these cyclers the measurement exposure must be adjusted. Choose the Operation with the device mode in the Settings menu, select the item Measurement exposition:

- FAM to 250
- HEX and ROX to 1000
- Cy5 to 500

Confirm that the current exposure value is saved by pressing YES

Attention! The specified exposure values are applicable only for RealLine kits and, if necessary, must be changed for other purposes.

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### Annex II: Programming and analysis of results using Rotor-Gene Cyclers

Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q (Qiagen, Germany)

#### Program real time PCR cycler:

- 1) Click **New** button.
- 2) Select **Advanced** template from the tab of the New Run wizard. Click **New** button.
- 3) Select **36-Well Rotor** type, check that No Domed 0.2 ml Tubes are used. Click **Next** button.
- 4) In the new window determine Reaction volume as **50 µl**. Click **Next** button.
- 5) The temperature profile of real time PCR should be set. Click **Edit Profile** button.

Step 1: Hold 1	45 °C	30 min	50 Cycles
Step 2: Hold 2	94 °C	1 min	
Step 3: Cycling	94 °C	10 sec	
	60 °C*	<b>40 sec</b>	
* measurement of fluorescent at 60 °C			

- 6) Then temperature profile is set, click **OK** button.
- 7) In the **New Run Wizard** window, click **Calibrate (Gain optimization)** button. The window **Auto Gain Calibration Setup** opens. In the line **Channel Settings** choose the **JOE, FAM, ROX,** and **Cy5** channels. Set the **Tube Position** to 1. Check **Perform Calibration Before 1st Acquisition**. Press the **Close** button.
- 8) Click **Next** button, start the amplification process by clicking **Start Run** button.  
Save a file in the Rotor-Gene/templates folder, named RealLine with \*.ret extension. In subsequent work RealLine template would be presented in New run wizard.
- 9) Save reaction result file with Rotor-Gene Run File \*.rex extension.
- 10) Record the positions of the controls and specimens according to the instruction manual of the cycler in use. Click **Start run** button.

#### Analysing the results of HCV cDNA amplification

- 1) Click **Analysis** button, choose **Quantitation** from the list, choose **Cycling A. Cy5 Red**, click **Show** button.
- 2) Click **OK** button, and cancel automatic **Threshold** determination.
- 3) Press the **Linear scale** button.
- 4) Make sure that, in the main window menu (*Quantitation analysis*), the two buttons, **Dynamic tube** and **Slope Correct**, are pressed.
- 5) Click the **More Settings** button, set the **NTC threshold 10 %** value.
- 6) In the **CT Calculation** area (*right side of the window*), set the **0.04** value in the **Threshold** window.
- 7) In the results table (*the Quant. Results window*), the **Ct** values will appear.

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### Analysing the results of genotype 1 HCV cDNA amplification

- 1) Press the **Analysis** button in the menu, select the **Quantitation** analysis mode, press the **Cycling A. JOE yellow** button, and then **Show**.
- 2) Cancel the automatic selection of **Threshold** by pressing **OK** in the pop-up.
- 3) Press the **Linear scale** button.
- 4) Make sure that, in the main window menu (*Quantitation analysis*), the two buttons, **Dynamic tube** and **Slope Correct**, are pressed.
- 5) Press the **More Settings** button, set the **NTC threshold 10 %** value.
- 6) In the **CT Calculation** area (*right side of the window*), set the **0.04** value in the **Threshold** window.
- 7) In the results table (*the Quant. Results* window), the **Ct** values will appear.

### Analysing the results of genotype 2 HCV cDNA amplification

- 1) Press the **Analysis** button in the menu, select the **Quantitation** analysis mode, press the **Cycling A. FAM green** button, and then **Show**.
- 2) Cancel the automatic selection of **Threshold** by pressing **OK** in the pop-up window.
- 3) Press the **Linear scale** button.
- 4) Make sure that, in the main window menu (*Quantitation analysis*), the two buttons, **Dynamic tube** and **Slope Correct**, are pressed.
- 5) Press the **More Settings** button, set the **NTC threshold 10 %** value.
- 6) In the **CT Calculation** area (*right side of the window*), set the **0.04** value in the **Threshold** window.
- 7) In the results table (*the Quant. Results* window), the **Ct** values will appear.

### Analysing the results of genotype 3 HCV cDNA amplification

- 1) Press the **Analysis** button in the menu, select the **Quantitation** analysis mode, press the **Cycling A. ROX orange** button, and then **Show**.
- 2) Cancel the automatic selection of **Threshold** by pressing **OK** in the pop-up window.
- 3) Press the **Linear scale** button.
- 4) Make sure that, in the main window menu (*Quantitation analysis*), the two buttons, **Dynamic tube** and **Slope Correct**, are pressed.
- 5) Press the **More Settings** button, set the **NTC threshold 10 %** value.
- 6) In the **CT Calculation** area (*right side of the window*), set the **0.04** value in the **Threshold** window.
- 7) In the results table (*the Quant. Results* window), the **Ct** values will appear.

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