# RealLine HCV Genotype quantitative Str-Format

Instructions for Use

# RealLine HCV Genotype quantitative Str-format

**REAL TIME PCR DETECTION AND DIFFERENTIATION KIT FOR HEPATITIS C VIRUS GENOTYPES 1, 2 AND 3 RNA WITH QUANTIFICATION**

For Research use only!

| | | |
|---|---|---|
| **RealLine HCV Genotype quantitative Str-format** | **VBD0797** | **48 Tests** |
| | | |
| **valid from** | **September 2019** | |

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## Explanation of symbols used in labeling

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

BIORON Diagnostics GmbH

In den Rauhweiden 20
67354 Römerberg
Germany

Phone +49 6232 298 44 0
Fax: +49 6232 298 44 29
info@bioron.de

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**HEPATITIS C VIRUS GENOTYPES 1, 2 AND 3 RNA REAL TIME PCR DETECTION AND DIFFERENTIATION KIT**

Research Use Only

## 1. INTRODUCTION

**RealLine HCV-Genotype** kit is intended for detection and assay of RNA and differentiation of 1/2/3 genotypes of Hepatitis C Virus by the method based on viral RNA reverse transcription followed by cDNA amplification in polymerase chain reaction (RT-PCR) with real-time fluorescence detection.

**RealLine HCV-Genotype** kit can be used to detect and determine viral load and Hepatitis C Virus genotype.

The kit is intended for use with the following equipment: iQ iCycler, iQ5 iCycler, CFX96 *(Bio-Rad, USA)*, DT-96 *(DNA-Technology, Russia) and RealLine Cycler (BIORON Diagnostics GmbH)*.

RNA extraction from clinical samples is carried out using the following kits:

- **RealLine Extraction 100 (VBC8896)**
- **RealLine Extraction 1000 (VBC8895)**

The kit is designed for analysis of 48 samples, including controls.

## 2. KIT CONTENTS

| | |
|---|---|
| Integrated Positive Control sample *(*PC), lyophilized | 1 vial |
| Ready Master Mix 1 **(RMM1)** for reverse transcription and PCR, lyophilized, **for genotypying HCV** | 48 test tubes (6 strips x 8 tubes) |
| Ready Master Mix 2 **(RMM2)** for reverse transcription and PCR, lyophilized, for **detection** and **quantitation** | 48 test tubes (6 strips x 8 tubes) |
| Recovery Solution for Control samples (RSC) | 1 vials, 4 ml each; |
| PCR adhesive foils | |
| Passport for the concentration of PC | |

## 3. PRINCIPLES OF THE METHOD

The principle of the method is based on reverse transcription reaction of a selected RNA fragment followed by cDNA amplification with real-time detection of PCR products.

Every sample undergoes analysis using two ready master mixes RMM-1 and RMM-2 included in kit. RMM-1 is intended for determining HCV RNA genotype, RMM-2 for HCV RNA assay in the test sample.

Determination of HCV RNA genotype is assured by independent detection of HCV genome fragments corresponding to various genotypes due to fluorogenic genotype-specific probes being detected in various channels. When determining a genotype, assay data are taken into account. To control RMM-1 performance in each analysis, **complex positive control (PC)** containing 1b, 2a, 3a HCV RNA genotypes are used.

PC is expressed in international units (IU/ml) per WHO standard *(WHO International Standard for Hepatitis C Virus NIBSC Code: 96/798)* and is the reference sample for calculating viral load. Determination of RNA viral amount in the initial sample is carried out by comparing the number of cycles required for reaching the threshold fluorescence for the test sample with characterised PC serving as the reference sample – **Ct**, additionally adjusted for RNA extraction efficacy and reverse transcription.

Efficiency control of RNA extraction from samples is ensured by HCV RNA extraction from clinical samples together with the pre-loaded **internal control (IC)**.

## 4. SPECIFICATIONS

### 4.1. Sensitivity.

The kit assures (*for 100% of samples*) HCV RNA detection in the concentration at least 15 IU/ml when extracting RNA from 1 ml of the sample.

The kit assures HCV genotype determination at RNA concentrations at least 400 IU/ml when extracting RNA from 1 ml of the sample.

### 4.2. Specificity.

The kit detects Hepatitis C virus of 1a, 1b, 2a, 2b, 2c, 2i, 3, 4, 5a, 6 genotypes regardless subtype, and determines genotypes 1 *(1a, 1b)*, 2 *(2a, 2b, 2c, 2i)*, 3 *(3a,3b)*. In samples containing HCV RNA (*above the limit of detection*), it determines HCV RNA concentration. In samples not containing HCV RNA, the analysis result shall be reliably negative (*for 100% of samples*).

**4.3. Range (linearity):** from 15 IU/ml to $10^8$ IU/ml of HCV RNA when extracting RNA from 1 ml of the sample.

To determine viral load in HCV RNA replicates per ml, ratio 1 IU / 2.5 replicates of HCV RNA should be used *(according to the data of National Institute of Biological Standards and Control for WHO international standard for Hepatitis C virus NIBSC Code: 96/798)*.

## 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.

## 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 DNA isolation and PCR test run must be spatially separated.
- Avoid microbial and ribonuclease 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.
- 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 kit.

## 7. ADDITIONAL MATERIALS AND DEVICES REQUIRED BUT NOT SUPPLIED

- NA extraction kits: **RealLine Extraction 100** or **RealLine Extraction 1000**.
- Real time PCR system;
- Laminar safety box;
- Refrigerator;
- Half-automatic variable-volume single-channel pipettes;
- Disposable medical non-sterile powder-free gloves;
- Disposable pipette tips with aerosol barrier;
- Biohazard waste container.

## 8. PREPARATION OF REAGENTS AND SPECIMEN

### 8.1 Specimen preparation

Prepare specimens for the assay with Extraction kit according to Extraction Kit manual.

*Every group of samples passing RNA extraction should contain 3 replicates of PC, which is a component of RT-PCR performance kit and negative control (NC) which is a component of RNA extraction kit*.

Prior to RNA isolation procedure add 1 ml of Recovery Solution for Control samples (RSC) into a vial with Integrated Positive Control (PC) sample, mix gently, keep for 15 minutes, then carefully mix once again.

*PC should be stored at (2 - 8) °C and used within 1 month of preparation.*

| |
|---|
| It is recommended to use three replicas of Positive Control sample and one Negative Control sample (NC) in each test run. |

### 8.2 Preparation of kit components

Prior to preparing samples, add **1 ml of RSC** to the vial with PC, mix carefully, close tightly the vial with a new plastic cap included in the kit, maintain at a room temperature for 15 minutes, then again carefully mix.
*Upon recovery, store the PC at (2–8) °C for not more than 1 month.*

Prior to getting started, remove the kit from the refrigerator, allow the RMM1 and RMM-2 in the package (*unopened*) to stay at (18–25) °C for at least 30 minutes.

Open the package, prepare any necessary amount (*according to the number of samples prepared including controls: 1 NC and 3 PCs*) of tubes with RMM-1 and RMM-2*. Pack the tubes remained unused with RMM-1 and RMM2 in ceflen bags with desiccator, remove excess air, and close the clamp tightly.

**The protective film of RMM-1 has green bar code and that of RMM-2 white bar code.*
*Upon first unpacking, RMM-1 and RMM-2 shelf life at a storage temperature (2–8) °C is 3 months.*

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## 9. PROCEDURE PROTOCOL

**9.1.** Place the tubes with samples prepared into a magnetic rack.

**9.2.** Number the tubes with RMM-1 and RMM-2 required for analysis *(according to the number of samples prepared, including controls)* and arrange in the rack.

**9.3.** Pipette **50 µl of the relevant RNA solution extracted** into each tube with RMM-1 and RMM-2 (using a filtered tip pipette).

**9.4.** Close the tubes and transfer the tubes into the thermocycler.

**9.5.** Program the device to perform amplification of a specific fragment of **HCV RNA and IC** and fluorescence signals detection.

**9.6.** Program real time PCR device as follows:

| | | |
|---|---|---|
| Stage 1: | 45°C, 30 min; | |
| Stage 2: | 94°C, 1 min; | |
| Stage 2: | 94°C, 10 sec | 50 cycles |
| | 60°C, 20 sec | |

*Fluorescence measurements should be done at 60°C.*

**9.7.** Select amplification detection channels

**RMM-1**

- For detection of amplification of **HCV genotype 1** cDNA: collect real-time PCR data through the **HEX** channel
- For detection of amplification of HCV genotype 2 cDNA: collect real-time PCR data through the **FAM** channel
- For detection of amplification of HCV genotype 3 cDNA: collect real-time PCR data through the **ROX** channel

**RMM-2**

- For detection of amplification of Internal control IC DNA: collect real-time PCR data through the **FAM** channel
- For detection of amplification of HCV cDNA: collect real-time PCR data through the **ROX** channel
- Record the positions of the controls and specimens according to the instruction manual of the operating device.

**9.8.** Start the PCR program.

## 10. DATA ANALYSIS

### 10.1 General conditions of analysis and reporting of results.

**10.1.1** For **complex PC,** the program should register as follows:

- **for RMM-1:** an increase of the specific signal of **HCV cDNA** amplification in **HEX** channel (*genotype 1*), **FAM** channel (*genotype 2*), **ROX** channel (*genotype 3*) and determine the **HCV Ct** threshold cycle value.

- **for RMM-2:** an increase of the specific signal of **HCV cDNA** amplification in **ROX** channel (*all genotypes*) and determine the **HCV Ct** threshold cycle value, an increase of the specific signal of **HCV cDNA** amplification in **FAM** channel and determine the **HCV Ct** threshold cycle value.

**10.1.2** Results of PCR individual performance are subject to analysis and reporting provided that for PC, the **Ct** value in **FAM**, **HEX**, **ROX** channels *(RMM-1)* and in **ROX** channel *(RMM-2)* are in the range indicated in the insert to the kits of this batch.

**10.1.3** For **NC <u>for RMM-1,</u>** the program should not register a reliable increase of the specific signal of **HCV cDNA** amplification in **FAM**, **HEX**, **ROX** channels. **<u>For RMM-2</u>**: the program should register an increase of **IC cDNA** amplification signal determine **IC Ct**, moreover, there should not be a reliable increase of the specific signal of **HCV cDNA** amplification.

If for NC, **the Ct value** in **FAM**, **HEX**, **ROX** channels *(RMM-1)* and **ROX** channel *(RMM-2)* **is less or equal to 40**, this evidences of **contamination in the system**. See the order of actions in Paragraph 9.2.4.

**10.1.4** For **<u>RMM-2,</u> in each test sample,** the program should register an increase of IC cDNA amplification signal *(FAM channel)* and determine IC **Ct**. The analysis result is considered reliable, if for this sample the **IC Ct** value in **FAM channel is less or equal to 40**.

### 10.2 Reporting of results.

**10.2.1.** Calculate (IC **Ct**)$_{av}$ as an average value of IC **Ct** of all test samples (*including PC and NC*). Values of IC **Ct** differing more than 2 from (IC **Ct**)$_{av}$ value are subject to rejection. Upon rejection, recalculate (IC **Ct**)$_{av}$ for the remaining values.

**10.2.2.** The test sample is considered to be negative (*free from HCV RNA*), if for this sample in RMM-2 the **Ct** value in **ROX** channel **is more than 40 or is not determined.**

If for such a sample, the IC **Ct** value exceeds that of (IC **Ct**)$_{av}$ more than 2, the result for this sample is not subject to reporting as negative. Reanalysis of this sample starting from the extraction stage is required. In case of result duplication, a repeated blood sample collection and reanalysis are recommended.

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**10.2.3.** The test sample is considered **positive**, i.e. containing HCV RNA, provided that the **Ct** value for this sample in RMM-2 in **ROX channel is less or equal to 40**. If for such sample the IC **Ct** value exceeds that of (IC **Ct**)$_{av}$ more than 2, the result for this sample is reported as positive without specifying RNA concentration. For assay, reanalysis of this sample starting from the extraction stage is required.

**10.2.4.** In case of contamination, all positive results obtained in this individual PCR performance should be considered unreliable. Measures related to detection and elimination of contamination sources are required as well as reanalysis of all samples in this performance for which **Ct values less than 40** in **FAM**, **HEX**, **ROX** channels *(RMM-1)* and in **ROX** channel *(RMM-2)* have been obtained. Samples of this performance, for which a negative result has been obtained, should be considered negative.

**10.3 Determination of HCV genotype**.

**10.3.1.** HCV genotype in the test sample is determined by comparing amplification results in two tubes *(RMM-1 and RMM-2)*.

**10.3.2.** The test sample **contains HCV, genotype 1**, if for **HEX** channel *(RMM-1)* and **ROX** channel *(RMM-2)* the **Ct value obtained is less than 40.**

**10.3.3.** The test sample **contains HCV, genotype 2**, if for **FAM** channel *(RMM-1)* and **ROX** channel *(RMM-2)* the **Ct value obtained is less than 40.**

**10.3.4.** The test sample **contains HCV, genotype 3**, if for **ROX** channel *(RMM-1)* and **ROX** channel *(RMM-2)* the **Ct value obtained is less than 40.**

**10.3.5.** If HCV RNA concentration in the test sample is less than 400 IU/ml, kit sensitivity may be insufficient for establishing HCV genotype.

**10.3.6.** If HCV RNA concentration in the test sample is more than 400 IU/ml *(RMM-2)*, and in RMM-1, **Ct value less than 40** is not registered in **FAM**, **HEX** or **ROX** channels, this means that this sample contains HCV RNA of genotype different from 1 *(1a, 1b),* 2 *(2a, 2b, 2c, 2i),* 3 *(3a,3b)*.

| | **RMM-1** | | | **RMM-2** | |
|---|---|---|---|---|---|
| **Channel** | **HEX** | **FAM** | **ROX** | **FAM** | **ROX** |
| **Detected cDNA** | HCV genotype 1 | HCV genotype 2 | HCV genotype 3 | IC | HCV *(all types)* |

**10.4 Assay of results.**

Assay is carried out based on the results of RMM-2 performance. For assay, calculate HCV RNA concentration in the test samples according to **Annex 1**

**10.4.1.** If the calculated value of HCV RNA concentration in the sample is within the range from 15 IU/ml to $10^8$ IU/ml, the result is determined as **positive** specifying the obtained HCV RNA concentration in the sample.

**10.4.2.** If calculated value of HCV RNA concentration is more than $10^8$ IU/ml, the result is recommended to be interpreted as **positive** with **HCV RNA concentration: "more than $10^8$ IU/ml".**

**10.4.3.** The test sample is reported as **negative** *(free from HCV RNA)*, provided that for this sample in RMM-2, **Ct** value in **ROX** channel **is more than 40 or is not determined.**

## 11. STORAGE AND TRANSPORTATION

- Store assay kit at (2 - 8) °C in the manufacturer's packing.
- Transport at (2 - 8) °C. Transportation at up to 25 °C for 10 days is allowed.
- Do not freeze reagents.
- Shelf life of the kit as printed on the label of the outside box.
- Do not pool reagents from different lots or from different vials of the same lot.
- Strictly follow the Instruction manual for reliable results.

## 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**

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: CALCULATION OF VIRAL RNA CONCENTRATION.**

Calculate the viral RNA concentration using the following equation:

$$C_{SAMPk} = C_{PC} \times 2^{(Ct\ PC - Ct\ SAMPk)} \times 2^{(Ct\ ICk - Ct\ ICPC)},$$

Where:

**k** – sample number;

$C_{PC}$ – PC concentration, specified in the passport of assay kit;

**Ct PC** and **Ct** $IC_{PC}$ – average Ct value for three **replicas of the PC samples ROX and FAM** channels, accordingly;

$Ct_{SAMPk}$ and **Ct** $IC_k$ – Ct value of the sample numbered k along **ROX** and **FAM** channels, accordingly, if amplification efficiency is **(Ea) =100%.**

For an easier calculation we can provide you with a Excel sheet. Ask us:
techsupport@bioron.de

***Note**: For precise calculation of viral NA concentration in analyzed samples, the validation of the analytical system should be done by comparison of **Positive Control PC** concentration considering Internal control concentrations. The PC concentration is specified in the passport of the assay kit. For this purpose three independent Positive Control samples are needed.*