

Instructions for Use

# RealLine HCV Quantitative - Uni-Format

QUANTITATIVE ASSAY KIT FOR THE EXTRACTION AND REAL TIME PCR DETECTION OF THE HEPATITIS C VIRUS RNA










**Attention!**  
Please read the information  
about quantification  
process carefully!

Research Use Only (RUO)

<b>RealLine HCV quantitative Uni Format incl. extraction</b>	<b>VBD0799</b>	<b>48 Tests</b>
<b>valid from:</b>	<b>November 2019</b>	

## RealLine HCV Quantitative 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
	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@biron.de

### Trademarks:

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

# RealLine HCV Quantitative Uni-Format

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## RealLine HCV Quantitative Uni-Format

### QUANTITATIVE ASSAY KIT FOR THE EXTRACTION AND REAL TIME PCR DETECTION OF THE HEPATITIS C VIRUS RNA

Research Use Only

#### 1. INTENDED USE

The assay kit **RealLine HCV quantitative** is intended for detection of hepatitis C virus (HCV) RNA in plasma and serum samples using the method of reverse transcription of viral RNA with subsequent cRNA amplification by real-time polymerase chain reaction (RT-PCR) with fluorescence detection of the amplified product.

The assay kit is designed for real-time PCR detection systems like iQ iCycler, iQ5 iCycler, CFX96 (Bio-Rad, USA), DT-96 (RNA-Technology, Russia); Rotor-Gene 3000, 6000 and Rotor-Gene Q (Qiagen, Germany), RealLine Cycler (BIORON Diagnostics GmbH) or their analogues.

The assay kit contains reagents sufficient for 4 x 12-test runs, which may be performed separately or simultaneously. It is strongly recommended to use one Positive Control sample and one Negative Control sample in each test run.

The assay kit can be used with either of two specimen preparation procedures, the Standard Procedure or the UltraSensitive procedure.

- In the Standard specimen preparation procedure, HCV RNA is isolated from **100 µl** of serum (plasma).
- In the UltraSensitive specimen preparation procedure, HCV viral particles in serum (plasma) are concentrated by Concentrating Solution of **1 ml** of serum (plasma).

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### 2. KIT CONTENTS

<b>Specimen Preparation Reagents:</b>	
<b>Concentrating Solution</b>	4 vials, 14 ml each
<b>Lysis Reagent № 1</b>	4 vials, 4 ml each
<b>Lysis Reagent № 2</b>	4 vials, 7 ml each
<b>Sorbent</b> (suspension of magnetic particles)	1 vial, 1 ml
<b>Solution for RNA/RNA Precipitation</b>	4 vials, 12 ml each
<b>Wash Solution № 1</b>	4 vials, 8 ml each
<b>Wash Solution № 2</b>	4 vials, 5 ml each
<b>Specimen Diluent</b>	4 vials, 3 ml each
<b>Control samples:</b>	
<b>Recovery Solution for Control samples (RSC)</b>	2 vials, 4 ml each
<b>Positive Control (PC)</b> , lyophilized	2 vials
<b>Negative Control (NC)</b> (Negative serum from human)	2 vials, 12 ml each
<b>Internal Control (IC)</b> , lyophilized	2 vials
<b>CS1 and CS2:</b> Samples for calibration, lyophilized - are used when the adequacy of analytical system has to be checked, see the Attachment 2	1 vial of each
<b>Amplification reagents</b>	
<b>Ready Master Mix</b> for reverse transcription and PCR, lyophilized (RMM)	48 test tubes
<b>Additional</b>	
The kit package also contains plastic caps for vials with control and calibration samples	6
Passport with the specific amounts for the controls in the upper lid of the box	

## RealLine HCV Quantitative Uni-Format

### 3. PRINCIPLE OF THE METHOD

The kit contains all reagents required for concentration and extraction of HCV RNA with subsequent quantification of HCV RNA by RT-PCR with fluorescence detection of the amplified product, taking into account the calibration and control samples.

Real-time PCR is based on the detection of the fluorescence produced by a reporter molecule, which increases as the reaction proceeds. Reporter molecule is a dual-labeled RNA probe that specifically binds to the target region of pathogen's cRNA, which is obtained from pathogen's RNA by reverse transcription. Fluorescence signal increases due to the separation of fluorescence dye and quencher owing to Taq RNA polymerase exonuclease activity during amplification. PCR consists of repeated cycles: temperature denaturation of cRNA, primer annealing, and complementary chain synthesis.

**Threshold cycle value (Ct)** is a cycle number at which the fluorescence generated within a reaction crosses the threshold and the fluorescence signal rises significantly above the background. Increased signal is due to the use of a RNA hybridization probe that is specific for the given cRNA sequence: it binds to one of the cRNA strands in the course of the reaction and provides additional specificity of the method. A RNA probe consists of a fluorescence dye at the 5'-end and a fluorescence quencher at the 3'-end that significantly reduces the fluorescence intensity. During the polymerase synthesis of the complementary strand, the probe is cleaved from the 5'-end due to the 5'-3' nuclease activity of Taq RNA polymerase, the quencher and the dye become separated, thus increasing the fluorescence signal due to the accumulation of the reaction product. The detected fluorescence intensity depends on the initial quantity of pathogen's cRNA template in the sample.

The use of **Internal Control sample (IC)** prevents the generation of false negative results associated with possible loss of RNA/cRNA template during sample preparation. IC indicates if PCR inhibitors occur in the reaction mixture. IC should be added to each sample (including control samples) prior to the NA extraction procedure. Amplification and detection of IC influence neither sensitivity nor specificity of the target cRNA PCR.

**Note:** *Internal Control sample is added to the sample during NA extraction step and is used throughout the whole process of NA extraction, amplification, detection.*

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### 4. SPECIFICATIONS

**Specificity:** The assay kit is designed for in vitro determination of HCV genotypes:

**1a, 1b, 2a, 2b, 2c, 2i, 3, 4, 5a, 6 regardless of subtype.**

The samples containing HCV RNA with concentration above the detection limit will be determined as positive. If specimen does not contain HCV RNA, analysis will give negative result (in 100 % of cases).

**Dynamic range** of estimated concentration (linearity area): from 15 IU / ml to 10<sup>8</sup> IE / ml HCV RNA for the UltraSensitive procedure (or from 150 IU/ml to 10<sup>8</sup> IU/ml for the Standard procedure).

1 IU = 2.5 copies of HCV RNA (*National Institute of Biological Standards and Control for WHO international standard for Hepatitis C virus NIBSC Code: 96/798*).

**Sensitivity:** The assay kit securely determines HCV RNA in concentration not less than 15 IU / ml for UltraSensitive specimen preparation procedure (or not less than 150 IU / ml for the Standard procedure).

### 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. WARNINGS AND SAFETY PRECAUTIONS

- ☞ For research use only. Not for use in diagnostic procedures. Any clinical diagnostic use is at the user's own risk and responsibility.
- ☞ When handling the kit, follow the national safety requirements for working with pathogens.
- ☞ To prevent contamination, the stages of RNA extraction and PCR test run must be spatially separated.
- ☞ Wear protective disposable gloves, laboratory coats, and eye protection when handling samples 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.
- ☞ To conduct an amplification reaction with real-time PCR products detection, use only disposable tips with filters.
- ☞ Never use the same tips for different samples.
- ☞ Do not pool reagents from different lots or from different vials of the same lot.
- ☞ Dispose of 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.

## RealLine HCV Quantitative Uni-Format

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

- Real time PCR device
- Laminar safety box;
- Microcentrifuge (min RCF 13000 rpm)
- Vortex mixer;
- Thermo Shaker
- Disposable gloves, powder-free;
- Half-automatic variable-volume single-channel pipettes with disposable tips;
- Disposable RNase/RNase-free tips with filters
- 2.0 ml polypropylene tubes, sterile, non-siliconised
- Vacuum aspirator;
- Racks for 2.0 ml and 0.2 ml tubes;
- Magnetic Rack for nucleic acids isolation.

### 8. SPECIMEN TRANSPORT AND STORAGE

Preparation of blood serum (plasma)

***Attention!*** *Samples anticoagulated with heparin are unsuitable for the test.*

Transfer serum (plasma) to a sterile polypropylene tube within 6 hours after blood sampling.

Transportation and storage of samples:

- At (18 – 25) °C – for no more than 6 hours;
- At (2 - 8) °C – for no more than 5 days
- At minus 18 °C and below – for a long time.

***Only one freeze-thaw cycle is allowed!***

Prior to use, centrifuge samples of serum (plasma) at (18 – 26) °C, 13,000 rpm for 5 min.



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### 9. REAGENT PREPARATION

**9.1.** Prior to work, take the assay kit from the refrigerator, open the package, and keep the kit components at room temperature (18 - 25) °C for 30 minutes.

**9.2.** Open the vial with **PC** by removing plastic cap and rubber stopper. Put the removed caps and stoppers into the container with disinfecting solution.

Add **1 ml of RSC**, tightly close the vial with new plastic cap provided with the kit. Carefully mix, keep at room temperature for 15 minutes, and then thoroughly mix again.

*After diluting store at (2–8)°C for no longer than 1 month.*

**9.3.** Open the vials with **CS1** and **CS2** by removing plastic caps and rubber stoppers. Put the removed caps and stoppers into the container with disinfecting solution.

Add **1 ml of RSC**, tightly close the vials with new plastic caps provided with the kit. Carefully mix, keep at room temperature for 15 minutes, and then thoroughly mix again

*Store diluted CS1 and CS2 store at (2 – 8) °C for no more than 1 month.*

**9.4.** Open the vial with **IC** by removing plastic cap and rubber stopper. Put the removed caps and stoppers into the container with disinfecting solution.

Add **1 ml of RSC**, tightly close the vial with new plastic cap provided with the kit. Carefully mix, keep at room temperature for 15 minutes, and then thoroughly mix again.

*Store diluted IC at (2 – 8) °C for no more than 1 month.*

**9.5.** Negative Control (NC) sample is ready to use.

**Attention!** *Once opened, NC should be stored at (2 - 8) °C and used within 1 month..*

**9.6.** Prior to use, heat **Lysis reagent № 1 and № 2** at (50 - 60) °C and mix thoroughly to dissolve the precipitated material. Vortex Sorbent to a condition of homogeneous suspension. Add **140 µl** of Sorbent suspension into a vial with Lysis Reagent № 2. Mix carefully.

**Attention!** *Lysis Reagent No. 2 is not to be stored after opening and adding Sorbent.*

For the quantitative determination, the RNA extraction is conducted from 100 µl of serum (plasma) using this kit. The Internal Control must be used with the Extraction, if another RNA Extraction kit is used, please add **RealLine Internal Control (REF VBC8881)** to the extraction procedure

Each set of samples must contain 3 PC and 1 NC sample.

The elution volume have to be 200 µl.

Please use for the NC samples the Negative Control Sample provided with the kit and do not use water or buffer.

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### 10. PROCEDURE PROTOCOL

**It is recommended to use three replicas of Positive Control sample and one Negative Control sample in each test run and to proceed the controls to the extraction procedure.**

#### 10.1. UltraSensitive procedure for RNA isolation: from 1 ml of serum (*plasma*)

**10.1.1** When choosing the number of test samples for RNA isolation ensure that each group of isolated samples and each individual PCR run contain 3 CS1, 3 CS2, 1 NC, and 1 PC.

***Attention!*** CS1 and CS2 undergo the RNA extraction procedure and RT-PCR during the first reaction with the kits of this lot and cyclers in use. Further RT-PCR test runs can be done using only two control points: NC and PC, the calibration plot can be imported from the first analysis.

**10.1.2** Prepare and label an appropriate number of 2 ml tubes for the test samples, PC, and NC.

**10.1.3** Pipette **30 µl of IC** to each tube.

**10.1.4** For NC, add to the tube, marked NC, **1 ml of Negative Control NC** sample.

**10.1.5** For PC, add to the tube, marked PC, **970 µl of NC**, and **30 µl of PC sample**

**10.1.6** For CS1 and CS2, pipette **970 µl of NC** and **30 µl** of the corresponding **calibration sample**

**10.1.7** Add **1 ml** of each specimen to the appropriately labeled tube.

**10.1.8** For each specimen or control tube, add **1 ml of Concentrating Solution**.

**10.1.9** Close the tubes with caps, thoroughly mix the contents by rolling over the tubes 5 times, and keep for 10–15 minutes at room temperature. Then spin at 3,000 rpm at room temperature for 5 min\*

\* The spinning parameters depend on the model of the centrifuge in use:

Eppendorf MiniSpin — 3,000 rpm;

Eppendorf 5415D — 2,000 rpm;

Eppendorf 5417C — 2,000 rpm; and

Heraeus BiofugePico — 2,000 rpm.

**10.1.10** Using a new tip for each sample, carefully remove the supernatant without disturbing the pellet. The pellet should be clearly visible at this step.

**10.1.11** Add **200 µl of Lysis reagent № 1** to each tube. Vortex vigorously for 10-15 seconds. Some insoluble material may remain. Leave tubes for 5 minutes at room temperature. Briefly spin to collect drops from the tube walls..

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- 10.1.12** Add **500 µl of Lysis reagent No. 2** with sorbent to each tube (with Lysis reagent No. 1). Vortex the contents of the tubes for 10–15 seconds. Keep in thermal shaker at 56°C for 10 min at 1,300 rpm. Briefly spin to dump collect from the tube walls.
- 10.1.13** Add **750 µl of NA precipitator** into each tube with the test samples.
- 10.1.14** Vortex the contents of the tubes for 10–15 seconds. Keep for 3–5 minutes at room temperature (18–25)°C. Spin for 5 minutes at 13,000 rpm.
- 10.1.15** Without shaking up the pellet place the tubes in the magnetic stand. Using pipette (*or aspirator*) with individual tip, remove supernatant from each tube without touching the pellet.
- 10.1.16** Add **500 µl of Wash Solution No. 1** to the pellet in each tube. Vortex the contents of the tubes for 10–15 seconds. Spin at 13,000 rpm for 5 min.
- 10.1.17** Without shaking up the pellet place the tubes in the magnetic stand. Using pipette (*or aspirator*) with individual tip, remove supernatant from each tube without touching the pellet.
- 10.1.18** Add **300 µl of Wash Solution No. 2** to the pellet in each tube. Vortex the contents of the tubes for 10–15 seconds. Spin at 13,000 rpm for 5 min.
- 10.1.19** Without shaking up the pellet place the tubes in the magnetic stand. Using pipette (*or aspirator*) with individual tip, remove supernatant from each tube without touching the pellet.
- 10.1.20** Dry the pellets in open tubes at room temperature (18 – 25) °C for 2–3 min.
- 10.1.21** Add **200 µl of Specimen Diluent** to the pellet in each tube. Thoroughly resuspend the pellet by vortexing. Incubate in thermal shaker at 56°C for 10 min at 1,300 rpm. Centrifuge at 13,000 rpm for 1 min. Samples are now prepared for a PCR test run.

***Attention!*** *Extracted RNA cannot be stored! Perform RNA extraction immediately before running the RT-PCR.*

### 10.2. Standard protocol for RNA isolation: from 100 µl of serum (plasma):

- 10.2.1** With small volume of test serum (*plasma*), the kit can be used to detect HCV RNA in **100 µl sample**. *Be aware that, in this case, detection of HCV RNA at concentrations lower than 50 IU/ml is not guaranteed.*
- 10.2.2** Pick out and sign the required number of **2 ml** tubes (*according to the number of test samples, including the required controls* — 3 CS1, 3 CS2, 1 NC, and 1 PC).
- 10.2.3** Pipette **30 µl of IC solution** into each tube.

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- 10.2.4** For the negative control, pipette **100 µl of NC** into the tube (*labelled as NC*).
- 10.2.5** For the positive control, pipette **70 µl of NC** and **30 µl of PC** into the tube (*labelled as PC*).
- 10.2.6** Pipette **70 µl of NC** and **30 µl** of the corresponding **calibration sample** into the tubes labelled as **CS1** and **CS2**.
- 10.2.7** In other tubes, pipette **100 µl of the test blood serum or plasma samples** according to the tube labels. Use individual pipette tip for each sample.
- 10.2.8** Add **500 µl of Lysis reagent No. 2 with sorbent** to each tube. Vortex the contents of the tubes for 10–15 seconds. Keep in thermal shaker at 56°C for 10 min at 1,300 rpm. Briefly spin to dump drops from the tube walls.
- 10.2.9** Add **600 µl of NA precipitator** into each tube with the test samples.
- 10.2.10** Vortex the contents of the tubes for 10–15 seconds. Keep for 3–5 minutes at room temperature (18 – 25) °C. Spin for 5 minutes at room temperature at 13,000 rpm.
- 10.2.11** Without shaking up the pellet place the tubes in the magnetic stand. Using pipette (*or aspirator*) with individual tip, remove supernatant from each tube without touching the pellet.
- 10.2.12** Add **500 µl of Wash Solution No. 1** to the pellet in each tube. Vortex the contents of the tubes for 10–15 seconds. Spin at 13,000 rpm for 5 min.
- 10.2.13** Without shaking up the pellet place the tubes in the magnetic stand. Using pipette (*or aspirator*) with individual tip, remove supernatant from each tube without touching the pellet.
- 10.2.14** Add **300 µl of Wash Solution No. 2** to the pellet in each tube. Vortex the contents of the tubes for 10–15 seconds. Spin at 13,000 rpm for 5 min.
- 10.2.15** Without shaking up the pellet place the tubes in the magnetic stand. Using pipette (*or aspirator*) with individual tips, remove supernatant from each tube without touching the pellet.
- 10.2.16** Dry the pellets in open tubes at room temperature (18 – 25) °C for 2–3 minutes.
- 10.2.17** Add **200 µl of Specimen Diluent** to the pellet in each tube. Thoroughly resuspend the pellet by vortexing. Incubate in thermal shaker at 56°C for 10 min at 1,300 rpm. Spin at 13,000 rpm for 1 min. Samples are now prepared for a PCR test run.

**Attention!** *Extracted RNA cannot be stored! Perform RNA extraction immediately before running the RT-PCR.*

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### 10.3. PCR

**10.3.1** Place the tubes with prepared samples and controls to the magnetic Rack.

**10.3.2** Prepare an appropriate number of reaction tubes with Ready Master Mix (RMM). Label each reaction tube for each specimen and control.

**Attention!** For blockcycler mark tubes on the lateral part, for Rotor-Gene mark on the cap.

**10.3.3** Add **50 µl** of corresponding extracted RNA solution and control to the appropriately labeled reaction tube using a new RNase-free tip with aerosol barrier for each sample. **Do not grasp sorbent particles!**

**10.3.4** Place reaction tubes into the thermal block of real time PCR device and program real time PCR device as follows:

**For iQ/iQ5 iCycler, CFX96, DT-96, RealLine Cycler:**

Step 1:	45°C	30 min	
Step 2:	94°C	1 min	
Step 3:	94°C	10 sec	50 Cycles
	60°C*	20 sec	
* measurement of fluorescent at 60 °C in FAM and ROX			

**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*	40 sec	
* measurement of fluorescent at 60 °C in FAM and ROX			

**10.3.5** Select the amplification detection channels:

- collect data through the **FAM** channel (iQ5 iCycler, CFX96, DT-96, Rotor-Gene 3000) or the **Green** channel (Rotor-Gene 6000, Rotor-Gene Q) for the detection of amplification signal of **IC cDNA**;
- collect data through the **ROX** channel (iQ5 iCycler, CFX96, DT-96, Rotor-Gene 3000) or the **Orange** channel (Rotor-Gene 6000, Rotor-Gene Q) for the detection of amplification signal of **HCV cDNA**

**10.3.6** Program the position of tubes with samples, calibration samples, positive and negative control samples according to the instruction manual to the used device.

**10.3.7** Run the program and perform PCR with fluorescent detection in real time.

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### 11. DATA ANALYSIS AND INTERPRETATION

Perform analysis, evaluation of results, and calculation of HCV RNA concentrations in the samples according to the instruction manual to the used device.

#### 11.1. General conditions of analysis and evaluation of results

11.1.1. In **PC**, the following should be registered:

- an increasing signal of the specific amplification product for HCV RNA (the **ROX (Orange)** channel) and determined the threshold cycle value, Ct, for HCV;
- an increasing amplification signal for IC DNA (in **FAM (Green)** channel) and determined the threshold cycle value, Ct, for **IC**.

11.1.2. The results of an individual RT-PCR run are to be analysed and evaluated if, for **PC**, the **HBV Ct** value is within the range indicated in the insert label for the kits of this lot.

11.1.3. For **NC**, an increasing amplification signal for **IC DNA** should be registered and **IC Ct** should be determined with no significant increase in the signal for the specific amplification product of **HCV RNA**. No **ROX (Orange)** fluorescence increase should appear.

11.1.4. For each **sample**, the program should detect the increase in the amplification signal of **IC cDNA** in **FAM (Green)** channel and determine **IC Ct**. The result of the test sample is considered valid if **IC Ct (FAM (Green))** is **less than or equal to 40**.

#### 11.2. Evaluation of results

11.2.1. Calculate  $(IC\ Ct)_{av}$  as an average value for **IC Ct** for all test samples (*including controls*). The **IC Ct** values differing by more than 2 from the  $(IC\ Ct)_{av}$  value should be discarded. After discarding, recalculate  $(Ct\ IC)_{av}$  for the remaining values.

11.2.2. The test sample is considered as **positive**, i.e. containing **HCV RNA**, if, for this sample, the **Ct** value in the **ROX (Orange)** channel is **less than or equal to 40**.

If, for such test sample, the **IC Ct** value differs from the  $(IC\ Ct)_{av}$  value by more than 2, such sample is evaluated as **positive** without indication of DNA concentration. For quantification, perform a repeated test for this sample starting from the isolation step.

11.2.3. The test sample is considered as **negative** (*not containing HCV RNA*) if, for this sample, the **Ct** value in the **ROX (Orange)** channel is **above 40 or not determined**.

If, for such sample, the **IC Ct** value exceeds the  $(IC\ Ct)_{av}$  value by more than 2, the result for this sample is not to be analysed and is evaluated as **negative**. A repeated test for this sample starting from the isolation step is required.

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In case of repetitive result take another blood sample and repeat the testing once again.

**11.2.4.** In case of contamination **all positive** results for this individual PCR run are **considered invalid**. Take measures to detect and eliminate the contamination source and repeat the testing for all samples of this run, for which the positive result has been obtained. The samples of this run, for which the test has yielded negative results, should be considered as negative.

### 11.3. Quantitative analysis of results

For quantification, calculate HCV RNA concentration in the test samples according to **Annex I**.

**Attention!** *If DNA was isolated from 100 µl of sample, multiply the result by 10.*

**11.3.1.** If the calculated value of HCV RNA concentration in the sample is within the range of 100 IU/ml to  $10^8$  IU/ml, the result is considered as **positive**, with indication of the obtained HCV RNA concentration in the sample (*in IU/ml*).

**11.3.2.** If the calculated value of HCV RNA concentration in the sample is higher than  $10^8$  IU/ml, the result is recommended to be interpreted as **positive**, with HCV RNA concentration “**greater than  $10^8$  IU/ml**”.

**11.3.3.** If the calculated value of HCV RNA concentration is less than 100 IU/ml, the result is recommended to be interpreted as **positive**, with HCV RNA concentration “**less than 100 IU/ml**”.

**11.3.4.** The test sample is considered **negative** (*not containing HCV RNA*) if, for this sample, if the Ct value in the **ROX (Orange)** channel is **above 40 or not determined**.

**11.3.5.** If the obtained concentration of PC is not within the range indicated in the insert label for the kit, repeat the tests for all samples from this run.

## 12. STORAGE AND TRANSPORTATION

- Store the assay kit at (2 - 8) °C in the manufacturer's packing.
- Transport at (2 – 8) °C. Transportation for up to 25 °C for up to 10 days is allowed.
- Do not freeze the kit!
- Do not pool reagents from different lots or from different vials of the same lot.
- Strictly follow the Instruction manual for reliable results.
- Do not use kits with damaged inner packages and get in contact with BIORON Diagnostics GmbH.

## RealLine HCV Quantitative Uni-Format

### ANNEX I: CALCULATING THE CONCENTRATION OF SPECIFIC VIRAL NA

#### Calculation of HCV RNA concentration

Quantitative analysis of HCV RNA is performed using the formulas below.

1) Calculate coefficient  $\beta$ :

$$\beta = [\lg(C_{CS1}) - \lg(C_{CS2})] / [\Delta Ct_{CS2} - \Delta Ct_{CS1}],$$

where  $C_{CS1}$  and  $C_{CS2}$  are the concentrations of specific viral NA in the calibration samples (*indicated in the insert label for the kit*);

$$\Delta Ct_{CS1} = Ct_{CS1av} - IC Ct_{CS1av};$$

$$\Delta Ct_{CS2} = Ct_{CS2av} - IC Ct_{CS2av};$$

$Ct_{CS1av}$ ,  $Ct_{CS2av}$  are the average  $Ct$  values for **CS1** and **CS2** samples through the “**ROX**” channel;  
 $IC Ct_{CS1av}$ ,  $IC Ct_{CS2av}$  are the average  $Ct$  values for **CS1** and **CS2** samples through the “**FAM**” channel.

2) Calculate the concentration of specific viral NA in the test sample ( $C_k$ ):

$$C_k = C_{CS2} \times 10^{\beta(\Delta Ct_{CS2} - \Delta Ct_k)},$$

where  $k$  is the sample number;

$$\Delta Ct_k = Ct_k - IC Ct_k$$

where  $Ct_k$  and  $IC Ct_k$  are the  $Ct$  values for the sample number  $k$  in the “**ROX**” and “**FAM**” channels, respectively.

If extraction and RT-PCR test run for **CS1** and **CS2** are not performed, use the  $\beta$  and  $\Delta Ct_{CS2}$  values obtained in the first test run with the kit of this lot for calculation.

For an easier calculation we can provide you with an Excel sheet. Ask us:

[techsupport@bioron.de](mailto: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.



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### ANNEX II: Programming the device and analysis of results using Rotor-Gene cyclers: Rotor-Gene 3000, Rotor-Gene 6000 (Corbett research, Australia), Rotor-Gene Q (Qiagen, Germany)

Hereinafter, detection channels and terms corresponding to different versions of devices and software are listed in the following order: Rotor-Gene 3000 (Rotor-Gene 6000, Rotor-Gene Q).

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

• Step 1:	• 50°C	• 2min	•
• Step 2:	• 95°C	• 2min	•
• Step 3:	• 94°C	• 10 sec	• 50 cycles
•	• 60°C*	• 40 sec	
- \* Measure the fluorescence at 60°C
- Then temperature profile is set, click **OK** button.
- In the **New Run Wizard** window click **Calibrate (Gain optimization)** button. The window **Auto Gain Calibration Setup** opens. In the line **Channel Settings** choose **ROX (Orange)**, click **Add**. Set **Tube Position 1**, **Min Reading 5**, **Max Reading 10**, click **OK**. In the line **Channel Settings** choose **FAM (Green)**, click **Add**. Set **Tube Position 1**, **Min Reading 5**, **Max Reading 10**, click **OK**.
- Tick off **Perform Calibration Before 1st Acquisition**. Click **Close** button.
- 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.
- Save reaction result file with Rotor-Gene Run File \*.rex extension.
- Record the positions of the controls and specimens according to the instruction manual of the operating device. Click **Start run** button.

### Results for Internal Control cDNA amplification

- Click **Analysis** button, choose **Quantitation** from the list, choose **Cycling A. FAM**, click **Show** button.
- Click **OK** button, and cancel automatic **Threshold** determination.
- Click **Linear scale** button.
- In the **Quantitation analysis** menu buttons **Dynamic tube** and **Slope Correct** should be pressed.
- Click **More Settings (Outlier removal)** button, determine **NTC threshold** value as **5%**.
- In the column **CT Calculation (right part of the window)** determine **Threshold** value as **0.04**.
- In the result table (**Quant. Results window**) **Ct** will be displayed.

## RealLine HCV Quantitative Uni-Format

### Results for HCV cDNA amplification

- Click **Analysis** button, choose **Quantitation** from the list, choose **Cycling A. ROX** click, **Show** button.
- Click **OK** button, and cancel automatic **Threshold** determination.
- Click **Linear scale** button.
- In the **Quantitation analysis** menu buttons **Dynamic tube** and **Slope Correct** should be pressed.
- Click **More Settings (Outlier removal)** button, determine **NTC threshold** value as **5%**.
- In the column **CT Calculation** (*right part of the window*) determine **Threshold** value as **0.04**.
- In the result table (**Quant. Results window**) **Ct** will be displayed.

### ANNEX III PROGRAMMING OF DT-96 CYCLER AND REALLINE CYCLER :

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

**FAM** to **500**;

**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, should be changed for other purposes.*

**RealLine HCV Quantitative  
Uni-Format**

RealLine Pathogen Diagnostic Kits

# RealLine HCV Quantitative Uni-Format

