

**RealLine HCV quantitative
Str-Format****Instructions for use****RealLine HCV quantitative
Str-Format****QUANTITATIVE KIT FOR REAL TIME PCR DETECTION FOR RNA OF HEPATITIS C VIRUS**










For research use only. Not for use in diagnostic procedures.

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

RealLine HCV quantitative (Str Format)	VBD0794	96 tests
valid from:	September 2019	

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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



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HEPATITIS C VIRUS RNA REAL TIME PCR DETECTION AND QUANTITATION KIT

Research Use Only

1. INTRODUCTION

The assay kit **RealLine HCV quantitative** is intended for the quantitative detection of hepatitis C virus (HCV) RNA in plasma and serum. The method is based on the reverse transcription of viral RNA to generate complementary DNA (cDNA), with subsequent amplification of target cDNA by Polymerase Chain Reaction (PCR) with fluorescent detection of amplified DNA in the real-time mode.

Assay kit is designed for use for real-time PCR detection systems like RealLine Cyclers (*BIORON Diagnostics GmbH*), iQ iCycler, iQ5 iCycler, CFX96 (*Bio-Rad, USA*), DT-96 (*DNA-Technology, Russia*) or their analogues.

The kit (VBD0794) contains reagents sufficient for 96 test runs.

The kit **RealLine HCV quantitative** is designed to detect HCV RNA isolated from serum (plasma) using the RNA extraction kits:

- **RealLine Extraction 100**
- **RealLine Extraction 1000.**

For the quantification process use three replicas of Positive Control sample and one Negative Control sample in each test run.

2. KIT CONTENTS

Ready Master Mix (RMM) for reverse transcription and PCR, lyophilized,	96 test tubes (12 strips x 8 tubes)
Positive Control sample (PC), lyophilized	2 vials
Weak Positive Control Sample (WPC HBV/HCV/HIV), lyophilized	1 vial
Recovery Solution for Control samples (RSC)	2 vials, 4ml each;
Calibrations sample 1 (CS 1 HCV). lyophilized	1 vial
Calibrations sample 2 (CS 2 HCV). lyophilized	1 vial
Adhesive Foils for PCR	
Passport for the concentration of Control PC, WPC, CS1 and CS2 (sticks inside the box)	

Attention! PC, WPC, CS1 and CS2 must undergo the RNA extraction procedure using RNA extraction kits: according instruction manual to the kit.

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3. PRINCIPLES OF THE PROCEDURE

The principle is based on the analysis of the detection process of the amplification of a specific fragment of cDNA (obtained by reverse transcription of HCV RNA), which consists in repeating cycles, thermal denaturation, annealing of primers to complementarity sequences, completion of the polynucleotide sequences with Taq-polymerase from these primers.

The detection method is based on measuring of fluorescence level in each PCR cycle. Increasing level of fluorescence is due to use of hybridization DNA-probe specific for given cDNA that binds in the reaction with one strand of cDNA also providing additional specificity. The DNA probe comprises a 5'-end fluorescent dye and the 3'-end - fluorescence quencher that significantly reduces fluorescence intensity. During the synthesis of the complementary strand polymerase probe is cleaved at the 5'-end due to the 5'-3' nuclease activity of Taq-polymerase, and the dye-quencher dissociating occurs resulting in the increasing of fluorescence level while accumulation of reaction product. The measured fluorescence intensity depends on the number of specific amplicons formed and dynamics of increase in fluorescence level is determined by the initial amount of cDNA in the sample.

The accuracy of the analysis is controlled by the presence of a positive result in the positive control sample (PC) and weak positive control (WPC) that undergo the procedure for RNA isolation together with analyzed samples.

Account of the effectiveness of the RNA isolation from the samples is provided by HCV RNA isolation from clinical samples together with the internal control sample (IC).

Note: IC is a component of the NA extraction kits of RealBest series. Internal Control is added to the sample during NA isolation 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).

Sensitivity

Assay kit securely determines HCV RNA in concentration not less than 15 IU/ml for the RNA isolation from 1 ml of serum (plasma).

The range of detectable concentrations: from 100 to 10^8 IU of HCV RNA in 1 ml of the sample.

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*).

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

- ☞ 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.
- ☞ 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

- Real time PCR system, like described in paragraph 1
- DNA-Extraction Kits: **RealLine Extraction 100** or **RealLine Extraction 1000**
- Internal Control reagent (VBC8881) and Negative Control Sample if the kit is used with the extraction kits of other supplier;
- Safety laminar 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;
- Razor or scalpel.

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8. PREPARATION OF THE ANALYSED SAMPLES AND REAGENTS

*Each group of samples undergoing the procedure of DNA isolation must include a **Positive Control sample (PC)** from this kit and a **Negative Control sample (NC)** which is a component of the DNA extraction kit.*

We strongly recommend the implementation of the Internal Control IC, the Negative Control NC and Positive Control PC samples to the extraction procedure, for quantitative determination use 3 Positive Control Samples.

When using a kit of another supplier for the extraction of nucleic acids as recommended in p1, add **20 µl** of **IC (VBC8881)** to each tube.

- For 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.

8.1 Sample preparation.

Prepare specimens for the assay with the extraction kit **RealLine Extraction 100** or **RealLine Extraction 1000** or according to Extraction kit manual. It is strongly recommended to use three replicas of Positive Control sample, one Weak Positive Control sample and one Negative Control sample in each test run.

Note: For the quantification process it is needed to perform three replicas of Positive Control sample and one Negative Control sample in each test run.

Attention: *If validation of the analytical system is necessary, the analysis should also include calibration samples 1 and 2 (CS1 and CS2) in three replicates each (see. Annex II).*

8.2 Preparation of the reagent

Prior to the test take the kit out of the refrigerator and keep the control samples and **Ready Master Mix for RT-PCR (RMM)** closed in the package at 18 - 25°C for at least 30 minutes. Then open the package and cut the necessary number of tubes in strips with RMM (*including prepared samples and controls: 1 NC, 1 WPC and 3 PC, when running together with calibrators – 3 CS1 and 3 CS2*) with the razor or scalpel.

Cut the tubes together with the covering film. Put the remaining strips immediately back into the foil pouch, squeeze the air out and tightly close with the clip.

Open the vials with PC and WPC by removing plastic cap and rubber stopper. Place the removed cap and stopper into the container with disinfectant. Add 1 ml of Recover Solution for Controls (RSC), close the vials tightly with plastic caps included in the kit. Gently mix and keep at room temperature for 15 min, then mix again thoroughly.

After dilution store at (2 – 8) °C for 1 month.

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9. PROCEDURE PROTOCOL

9.1. Label each reaction tube for each specimen and control sample.

Attention! For RealLine Cyclers, iQ/iQ5 iCycler and CFX96 put marks on the lateral part of a reaction tube.

9.2. Add **50 µl** of corresponding isolated RNA solution to each tube using a separate pipette tip with filter. *Do not grasp sorbent particles!* Tightly close the tubes with caps or seal with the PCR transparent film

9.3. Place the tubes into the thermal block of real time PCR device.

9.4. Program real time PCR device as follows

Stage 1:	45°C, 30 min;	
Stage 2:	94°C, 1 min;	
Stage 3:	94°C, 10 sec] 50 cycles
	60°C, 20 sec	

* measurement of fluorescence at 60 °C.

9.5. Select the amplification detection channels:

- Collect Real-time PCR data through the **FAM** channel for detection of amplification of **IC DNA**.
- Collect Real Time PCR data through the **ROX** channel for detection of amplification of **HCV RNA**.

9.6. 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.

9.7. Run the program.

NOTE: 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**
- **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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10. DATA ANALYSIS

10.1 In **Positive Control** sample and **Weak Positive Control** sample (also for **CS1** and **CS2**) the program should detect:

- **ROX** fluorescent signal increase and Ct value (HCV cDNA amplification);
- **FAM** fluorescent signal increase and Ct value (IC cDNA amplification).
-

The results of the individual PCR run are subject to analysis and accounting when HCV Ct value for PC is in the range specified in the insert for kits of this lot.

10.2 In **Negative Control** sample the program should detect a **FAM** fluorescent signal increase and Ct value, and no significant **ROX** fluorescent increase should appear.

If Ct value for NC along **ROX** channel is less than 40, this indicates the presence of contamination.

10.3 The program should detect an amplification signal increase for IC cDNA (channel **FAM**) in each sample and define Ct for IC. Probe analysis is valid if Ct of IC for this sample is equal to or less than 40.

10.4 Calculate $(IC\ Ct)_m$ as the average Ct value of IC for all samples (including PC and NC). Samples with Ct of IC, that differ from $(IC\ Ct)_m$ by more than 2, should be ignored. After screening, recalculate $(IC\ Ct)_m$ for remaining samples.

10.5 The sample is considered **negative** if Ct value via the **ROX** channel **exceeds 40 or is not determined**.

10.6 If Ct of IC for this sample differs from $(IC\ Ct)_m$ by more than 2, then result for this sample should be considered as equivocal. The test should be repeated from the sample RNA extraction stage.

10.7 The sample is considered **positive** if Ct value via the **ROX** channel **does not exceed 40**. If Ct of IC for this sample differs from $(IC\ Ct)_m$ more than 2, the sample is considered as positive without quantitative analysis. For quantitative analysis, repeat the test beginning from the RNA isolation stage.

If IC Ct value for such sample differs from the $(IC\ Ct)_{av}$ value by more than 2, then the sample is considered positive without stating RNA concentration. For quantitative analysis, it is necessary to repeat the analysis of the sample, starting with the isolation step.

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

10.9 If HCV Ct for WPC is above 40 or not determined, all negative results are considered invalid and require a repeated analysis.

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Summary of results:

	FAM IC	ROX HCV	Test	Results
PC	+	+	Valid	
	-	-	Not valid	Repeat test
	+	-	Not valid	Repeat test
	-	+	Questionable	Check results, possibly repeat tests
NC	+	-	Valid	
	-	-	Not valid	Repeat test
	+	+	Not valid	Repeat test, contamination
	-	+	Not valid	Repeat test
Samples	+	Ct < 40	Valid	Positive
	+	- Or Ct > 40	Valid	Negative
	-	-	Not valid	Repeat test
	-	+	Questionable	Check results, possibly repeat test

10.10 HCV quantitation analysis.

For quantitative analysis calculate the HCV RNA concentration in analyzed samples in accordance with Annex I.

Attention! When isolating RNA from 1 ml of sample, the obtained value corresponds to the concentration of HCV RNA in IU/ml. If RNA isolation was performed from a smaller volume sample, the value must be multiplied by ω , where $\omega = 1 / \text{sample volume in ml}$. Thus HCV RNA concentration is obtained, expressed in IU/ml.

- If calculated HCV RNA concentration is in dynamic range from 100 IU/ml to 10^8 IU/ml result should be reported as positive with indication of calculated HCV RNA concentration in the sample (in IU/ml).
- Test results higher than 10^8 IU/ml are above the upper limit of quantitation of the Standard test and should be reported as **higher than 10^8 IU/ml**.
- Test results less than 100 IU/ml are below the lower limit of quantitation of the Standard test and should be reported as HCV RNA detected, **less than 100 IU/ml**.
- The analyzed sample is considered as negative if it obtains no **Ct** value along the **ROX** channel or **Ct** value exceed 40.

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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.
- 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.

For further information and help, ask us at techsupport@bioron.de. We can provide you a calculation sheet for an easy evaluation of your data.

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ANNEX I: The calculation of the concentration of a specific viral NA

1. For samples PC Ct value of which is in the range of values, indicated in the insert to the kits of this lot, calculate the corrected HCV Ct values with the introduction of amendments to the effectiveness of extraction, controlled by IC Ct, according to the formula:

$$Z_{PC} = Ct_{PC} + [(IC\ Ct)_{av} - IC\ Ct]$$

Where:

Z_{PC} - corrected value HCV Ct for PC sample;

$(IC\ Ct)_{av}$ — average IC Ct, calculated for all samples (*see instruction manual*);

PC Ct and IC Ct - Ct values of the given PC sample via the channels **ROX** and **FAM**, respectively.

2. Calculate the mean value of the corrected threshold cycle for PC (Z_{PCav}).
 Z_{PC} values that differ by more than 2 from the Z_{PCav} should be ignored. Recalculate the Z_{PCav} for the remaining values after the screening.

3. Calculate the quantity of HCV RNA in each test sample (Q_k) using the formula:

$$Q_k = 10^{X_k} [IU],$$

Where:

$$X_k = A + B \times (Z_{PCav} - Z_k);$$

A - coefficient, defined as the logarithm of the quantity of HCV RNA in PC, given in the certificate to the lot: $A = \lg(Q_{PC})$;

Z_{PCav} - the average value Z_{PC} for the PC samples;

k - sample number;

Z_k - corrected Ct value of the sample, calculated as follows:

$$Z_k = HCV_k\ Ct + [(IC\ Ct)_{av} - (IC\ Ct)_k];$$

B – coefficient, dependent on amplification efficiency and calculated according the formula:

$$B = \lg(2 \times Ea/100),$$

Where Ea – efficiency of amplification in %. For quantitative estimation use $B=0.3$

4. Calculate the concentration of HCV RNA C_k (in IU/ml) in each of the studied sample:

$$C_k = Q_k / V_k$$

where V_k - is the volume of the sample in ml.

Using the above procedure, a concentration of HCV RNA in the given sample expressed in IU/ml is obtained.

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ANNEX II: VALIDATION OF THE ANALYTICAL SYSTEM.

Determining the concentration of a specific viral NA in IC using the calibration curve.

For a more accurate determination of the specific viral NA concentration in samples it is necessary to validate the analytical system by comparing passport concentrations of specific viral NA in IC with the value obtained from the calibration graph.

Prior start, extract the extraction kit and assay kit for detection and quantitative determination of HCV RNA from the refrigerator, open the packaging and incubate components of the kit at room temperature (18 - 25) °C for at least 30 min.

Prepare the kit components and samples NC, PC and WPC (see. Instruction manual).

Add 1 ml of recovery solution for controls (RSC) into vials CS1 and CS2, mix gently, keep at room temperature for 15 minutes, then again mix thoroughly.

Prepare 11 tubes according the instruction manual and label them: 1 tube NC, 1 tube WPC, 3 tubes of PC, CS1 and CS2 each.

Add control samples to appropriately labeled tubes according the instruction manual.

Perform RNA isolation according to instruction manual.

For isolated specimen prepare samples for PCR or RT-PCR, program the device, in accordance with an instruction to a device select concentrations of CS1 and CS2 specified in the device passport, and conduct the PCR or RT-PCR.

For each sample, calculate the corrected value of Ct by the formula:

$$Z = \text{HCV Ct} + [(\text{IC Ct})_{\text{av}} - \text{IC Ct}].$$

Calculate the mean value of Z for PC, CS1, CS2 (Z_{PCav} , Z_{CS1av} , Z_{CS2av}).

Discard the results of analysis for samples for which Z differs by more than 2 from Z_{av} . Calculate Z_{av} for the remaining samples.

Calculate B - coefficient for calculating the concentration of the specific viral NA, using the formula:

$$B = [\lg(Q_{\text{CS1}}) - \lg(Q_{\text{CS2}})] / (Z_{\text{CS2av}} - Z_{\text{CS1av}})$$

where:

Q_{CS1} and Q_{CS2} - concentrations of a specific viral NA in the calibration samples 1 and 2 (specified in the certificate for the kit);

When the amplification efficiency is 100% **$B = \lg 2 = 0.3$** .

According to the analysis results of calibration samples calculate the concentration of HCV RNA in PC using analytical formula:

$$Q_{\text{PC}} = 10^{X_{\text{PC}}} [\text{IU}],$$

where:

$$X_{\text{PC}} = \lg(Q_{\text{CS1}}) + B \times (Z_{\text{CS1av}} - Z_{\text{PCav}});$$

Z_{CS1av} - average value of Z for the CS1 sample;

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Z_{PCav} - the average value of Z for the PC samples;

When the amplification efficiency is 100% **B = lg2 = 0.3**.

The use of PC as a reference sample in further calculations of specific viral NA concentration in samples is allowed, if the calculated value of the specific viral NA concentration in PC differs no more than 2 times from the value specified in the certificate to the kit.

When all of these conditions are met, calculations of HCV RNA (in IU) in the samples can be performed using the value of the coefficient B calculated from the calibration graph:

$$Q_{PC} = 10^{X_{PC}} \text{ [IU]},$$

Where:

$$X_k = \lg(Q_{PC}) + B \times (Z_{PCav} - Z_k);$$

Concentration of HCV RNA C_k in each of the studied sample is calculated according the formula:

$$C_k = Q_k / V_k$$

where V_k - is the volume of the sample in ml.

Using the above procedure, concentration of HCV RNA in the given sample expressed in IU/ml is obtained.

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