Instructions for Use

RealLine HBV / HCV / HIV Str-Format

ASSAY KIT FOR THE QUALITATIVE AND DIFFERENTIAL DETECTION OF HEPATITIS B VIRUS DNA, HEPATITIS C VIRUS RNA, AND HUMAN IMMUNODEFICIENCY VIRUS TYPES 1 AND 2 RNA USING THE PCR/RT-PCR METHOD IN REAL TIME

For Research Use Only!

RealLine HBV / HCV / HIV (Str-Format)	**VBD0592**	**48 Tests**
valid from	**January 2020**	

BIORON Diagnostics GmbH)
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RealLine HBV / HCV / HIV Str-Format

Explanation of symbols used in labeling

RUO	For research use only
LOT	Batch code
REF	Catalogue number
	Content of number of tests
	Expiry date
	Temperature limitation
	Consult instructions for use
	Manufacturer
	Keep out of sunlight

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ASSAY KIT FOR THE QUALITATIVE AND DIFFERENTIAL DETECTION OF HBV, HCV AND HIV TYPES 1 AND 2 RNA USING THE PCR/RT-PCR METHOD IN REAL TIME

For research use only

1. INTRODUCTION

The **RealLine HBV/HCV/HIV PCR** assay kit is intended for differential detection of hepatitis B virus (HBV) DNA, hepatitis C virus (HCV) RNA, and human immunodeficiency virus types 1 and 2 *(HIV-1 and HIV-2)* RNA in blood serum *(plasma)* using a method based on the DNA or cDNA fragment *(obtained from RNA by reverse transcription)* amplification in polymerase chain reaction (PCR) with hybridization fluorescent detection in real time.

Nucleic acids isolation from blood serum (*plasma*) or other samples is performed using one of the following kits

RealLine Extraction 100 (REF VBC8896)
RealLine Extraction 1000 (REF VBC8895)

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

The **Str-Format Kit** contains 2 Mastermixes with 48 tubes (0.2 ml) in strips with lyophilized Ready-for-use Mastermix. 50 µl of extracted DNA have to be pipetted into the tube and the ready mastermix is diluted. The kit contains reagents required for 48 tests, including control samples and the positive control sample.

The use of:

- **Extraction Kits for nucleic acids from clinical specimen from other supplier**
- **other real-time PCR devices**
- **appropriate reaction volumes, other than 50 µl**

has to be validated in the lab by the user. The special notes regarding the internal control IC have to be strongly followed.

2. KIT CONTENTS

Positive Control Sample *(*PC) containing HBV DNA, HCV RNA, HIV-1 RNA, and HIV-2 RNA	1 vial lyophilized
Recovery Solution for Controls (RSC)	1 vial, 4 ml
Ready Master Mix (RMM), for PCR/RT-PCR No. 1 (RMM-1), for differential detection of HBV DNA and HCV RNA	48 test-tubes (6 strips x 8 tubes) lyophilized
Ready Master Mix (RMM), for RT-PCR No. 2 (RMM-2), for differential detection of HIV-1 RNA and HIV-2 RNA	48 test-tubes (6 strips x 8 tubes) lyophilized

The kit is additionally supplied with optical-transparent PCR-film and a screw cap for the PC after dilution.

3. PRINCIPLE OF THE METHOD

The assay methodology is based on recording the process of amplification of a selected specific DNA or cDNA fragment (*obtained from RNA by reverse transcription*) that involves repeating cycles of: temperature denaturation, annealing of primers with complementary sequences, and extension of polynucleotide sequences from these primers with Taq polymerase.
Differential detection of viral infections is ensured by an independent detection of hepatitis B and C viruses' genome fragments (in RMM-1) and human immunodeficiency viruses genome fragments corresponding to types 1 and 2 (in RMM-2) using specific fluorogenic probes detected in different channels.

The assay reliability is controlled by the presence of the positive result in the PC (Positive Control), which is subjected to the nucleic acids (NAs) isolation procedure along with the tested samples.
Evaluation of the efficiency of NAs isolation from samples is ensured by nucleic acids isolation from the clinical specimens along with the **internal control sample (IC)**.

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4. SPECIFICATIONS

4.1. Specificity of HBV DNA and HCV RNA detection is determined using the samples containing no HBV DNA and HCV RNA but containing HIV-1 RNA and HIV-2 RNA. Specificity is 100% provided that all prepared samples are determined as negative.

4.2. Specificity of HIV-1 RNA and HIV-2 RNA detection is determined using the samples containing no HIV-1 RNA and HIV-2 RNA but containing HBV DNA and HCV RNA. Specificity is 100% provided that all prepared samples are determined as negative.

4.3. Sensitivity of HBV DNA and HCV RNA detection is determined using five samples, containing HBV DNA (*10 IU/ml*) and HCV RNA (*15 IU/ml*) prepared with the respective HBV DNA SOP and HCV RNA SOP. Sensitivity is 100% provided that all prepared samples are determined as positive.

4.4. Sensitivity of HIV-1 RNA and HIV-2 RNA detection is determined using five samples, containing HIV-1 RNA (*30 IU/ml*) and HIV-2 RNA (*50 IU/ml*) prepared with the respective HIV RNA SOP and HIV-2 RNA SOP. Sensitivity is 100% provided that all prepared samples are determined as positive.

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

WARNING! Failure to comply with the following requirements can lead to distortion of PCR results.

- 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 NA 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. ADDITIONAL MATERIALS AND DEVICES REQUIRED BUT NOT SUPPLIED

- real time PCR system, like described in p.1
- DNA-Extraction Kit: 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;
- laminar safety box;
- refrigerator;
- vortex;
- half-automatic variable-volume single-channel pipettes;
- disposable medical non-sterile powder-free gloves;
- disposable pipette tips with aerosol barrier;
- stands for tubes (0.2 ml, 1.5 ml or 2 ml)
- biohazard waste container
- magnetic stand for tubes
- 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.

When using a kit of another supplier for the extraction of nucleic acids as recommended in chapter 1, add **20 µl** of **IC (VBC8881)** to 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.

8.1. Sample preparation

The assay is performed on samples like blood serum (plasma), from which NAs have been preliminarily isolated using one of the following kits: **RealLine Extraction 100** or **RealLine Extraction 1000** according their instruction manuals.

8.2. Preparation of the reagents.

Prior to work, take the kit from the refrigerator, leave RMM and other kit components in their original packaging (*do not open*) at the temperature of (18 – 25) °C for 30 minutes.
Carefully open the RMM *(RMM-1 and RMM-2)* package using a knife or razor, cut off the required number *(according to the number of the prepared specimens, including control samples — 1 NC and 1 PC*) of tubes containing RMM-1 and the same number of tubes containing RMM-2. Cut the tubes off together with the film covering them.
Put the unused RMM tubes back into the foil with desiccant, squeeze the air from the pouch, and tightly close the clamp.
After initial opening the shelf life of RMM is 3 months at (2 – 8) °C.

8.3. Preparing PC for NA isolation

Open the vial with PC by removing the plastic cap and rubber stopper. Put the removed cap and stopper into the container with disinfectant. Add **1 ml of RSC**, tightly close the vial with a new plastic cap provided with the kit. Carefully mix, keep at the temperature of (18 – 25) °C for 15 minutes, and then thoroughly mix again.
After reconstitution store at (2 – 8) °C for no longer than 1 month.

9. PROCEDURE

9.1. Place the tubes with the prepared specimens in the magnetic stand.

9.2. Enumerate the required number (according to the number of the prepared specimens, *including control samples*) of the tubes containing RMM-1 and place them in the stand.

9.3. Enumerate the required number (*according to the number of the prepared specimens, including control samples*) of the tubes containing RMM-2 and place them in the stand.

Warning! *Place inscriptions on the tube sides.*

9.4. Into each tube with RMM-1 and RMM-2, add **50 µl** of the relevant **solution of isolated NA** *(from the tubes placed in the magnetic stand)* using a pipette with the individual filter tip. Do not touch sorbent particles!

9.5. Seal tubes tightly with optical film. Place the tubes in the mini-shaker. Mix the content of the tubes at 1,800 rpm for 1 minute.

9.6. Place the tubes into the thermal cycler.

9.7. Program the device to perform reverse transcription followed by amplification of specific fragments of **HBV DNA, HCV cDNA, HIV-1 cDNA, HIV-2 cDNA and IC cDNA,** and to detect the fluorescence signals.

9.8. Program the RT-PCR protocol.

Stage 1:	**50°C, 30 min**	
Stage 2:	**94 °C , 1 min**	
Stage 3:	**94 °C, 10 sec**	**50 cycles**
	60°C*, 60 sec	

* Measure the fluorescence at 60°C.

Note: the protocol containing the preliminary step of reverse transcription is not influencing the HBV DNA fragment amplification.

9.9. To detect signal during the amplification of the **IC cDNA** fragment, both in RMM-1 and RMM-2 the hybridization DNA probe labelled with fluorophore FAM is used.

- Select the **FAM** detection channel to provide detection.

To detect signal during the amplification of the **HBV DNA** fragment in RMM-1 and **HIV-1 cDNA** in RMM-2, the hybridization DNA probes labelled with fluorophore ROX are used.

- Select the **ROX** detection channel to provide detection.

To detect signal during the amplification of the **HCV cDNA** fragment in RMM-1 and **HIV-2 cDNA** in RMM-2, the hybridization DNA probes labelled with fluorophore HEX are used.

- Select the **HEX** detection channel to provide detection.

9.10. 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.

9.11. Run the program.

10. DATA ANALYSIS AND INTERPRETATION

10.1. Conditions of the results analysis

10.1.1. In **PC**, register the following:

- increasing signal of the specific **HBV DNA** amplification product (***RMM-1 - ROX channel***) and determine the value of the **HBV Ct** threshold cycle;

- increasing signal of the specific **HCV cDNA** amplification product (***RMM-1 - HEX channel***) and determine the value of the **HCV Ct** threshold cycle;

- increasing signal of the specific **HIV-1 cDNA** amplification product (***RMM-2 - ROX channel***) and determine the value of the **HIV-1 Ct** threshold cycle;

- increasing signal of the specific **HIV-2 cDNA** amplification product (***RMM-2 - HEX channel***) and determine the value of the **HIV-2 Ct** threshold cycle;

- increasing **IC cDNA** amplification signal (***RMM-1, RMM-2 - FAM channel***) and determine the value of the **IC Ct** threshold cycle.

10.1.2. In **NC**, an increasing amplification signal for **IC cDNA** should be registered and **IC Ct** should be determined with no increase in signal of the specific **HBV DNA, HCV cDNA, HIV-1, and HIV-2 amplification products.**

If the **Ct** value for NC for at least one of the causative agents is **less than or equal to 40**, this indicates the **presence of contamination** in the system. In this case, follow the procedure in 9.2.7.

10.1.3. For each test sample, record an increasing **IC cDNA** fragment amplification signal **(FAM channel)** and determine the **IC Ct.** The specimen assay is considered valid, if IC Ct for this sample in the FAM channel is less than or equal to 40.

10.2. Results evaluation

10.2.1. Calculate **(IC Ct)$_{av}$** as the average IC Ct value for all test sample (including PC and NC) for each RMM. The IC Ct values differing by more than 2 from the (IC Ct)$_{av}$ value should be discarded.

10.2.2. The test sample is evaluated as **negative** (*containing no HBV DNA, HCV, HIV-1, HIV-2* RNAs), when the **Ct** value for this sample in RMM-1 and RMM-2 via the **ROX** and **HEX** channels is **more than 40 or is 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 evaluated as negative. A repeated assay for this sample starting from the isolation step is required. In case of repetitive result take another blood sample and repeat the assay once again.

10.2.3. The test sample is considered **positive** on **HBV** *(containing HBV DNA)*, when the **Ct** value for this sample in **RMM-1** in the **ROX channel is less than or equal to 40**.

10.2.4. The test sample is considered **positive** on **HCV** *(containing HCV RNA)*, when the **Ct** value for this sample in **RMM-1** in the **HEX channel is less than or equal to 40**.

10.2.5. The test sample is considered **positive** on **HIV-1** *(containing HIV-1 RNA)*, when the **Ct** value for this sample in **RMM-2** in the **ROX channel is less than or equal to 40**.

10.2.6. The test sample is considered **positive** on **HIV-2** *(containing HIV-2 RNA)*, when the **Ct** value for this sample in **RMM-2** in the **HEX channel is less than or equal to 40**.

10.2.7. 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 assay 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.

If in **PC** the **Ct** value for at least one of the causative agents is **more than 40 or is not determined**, then all negative results for this causative agent are considered invalid and are required to be run again.

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11. STORAGE AND TRANSPORTATION

- Store and transport the assay kit at (2 - 8) °C in the manufacturer's packing.
- Transportation 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.

- **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 (RMM): 3 months at (2 – 8) °C.

Technical Support: techsupport@bioron.de

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.