Instructions for Use

RealLine HIV quantitative Str-Format

DETECTION AND QUANTIFICATION OF THE HUMAN IMMUNODEFICIENCY VIRUS RNA BY REAL TIME PCR

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

Attention!
Please read the information about quantification process carefully!

RealLine HIV quantitative (Str-format)	**VBD0195**	**96 Tests**
valid from	**September 2019**	

BIORON Diagnostics GmbH
info@bioron.de - www.bioron.de

RealLine HIV quantitative Str-Format

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

RealLine HIV quantitative Str-Format

Table of content:

RealLine HIV quantitative Str-Format

HUMAN IMMUNODEFICIENCY VIRUS (HIV) RNA REAL TIME PCR DETECTION AND QUANTIFICATION

Research Use Only

1. INTRODUCTION

Assay kit **RealLine HIV Quantitative** kit is intended for the quantitative detection of Human Immunodeficiency virus (HIV) 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 adapted 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 assay kit contains reagents sufficient for 96 test runs.

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

RealLine HIV is designed to detect HIV RNA isolated from serum *(plasma)* using RNA extraction kit: **RealLine Extraction 100** or **RealLine Extraction 1000**.

2. KIT CONTENTS

Ready Master Mix for reverse transcription and PCR, freeze-dried (RMM)	96 test tubes (12 strips × 8 tubes)
Recovery Solution for Control samples (RSC)	2 vials 4 ml each
Weak Positive Control Sample (WPC HBV/HCV/HIV), freeze-dried	1 vial
Positive Control (PC) sample, freeze-dried	2 vials
Samples for calibration Calibration Sample No 1 (CS1) Calibration Sample No 2 (CS2) freeze-dried *are used when the adequacy of analytical system has to be checked, see the Attachment 2*	1 vial each
Passport with the specific concentrations of controls	

3. PRINCIPLES OF THE PROCEDURE

Principle of analysis is based on the reverse transcription of viral RNA with subsequent PCR amplification of target cDNA by PCR with fluorescence detection of amplified DNA in the real-time mode.
Reliability of analysis is provided by application of Weak Positive Control sample.

Quantification of Human Immunodeficiency virus RNA is provided by application of the Positive Control (PC) in each test run. Positive Control is characterized in international units (IU/ml) by World Health Organization *(WHO international standard for Human Immunodeficiency virus NIBSC Code: 97/656)*, and serves as a Quantitation Standard for calculation of viral quantity.

Threshold cycle value – **Ct** – is the cycle number at which fluorescence generated within a reaction crosses fluorescence threshold, a fluorescence signal significantly above the background fluorescence. Quantity of viral RNA in initial sample is calculated by comparison of the threshold cycle value of analyzed sample and Positive Control. Also efficiency of sample preparation and reverse transcription should be considered.

4. SPECIFICATION

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

Dynamic range of estimated concentration (linearity area): from 20 IU/ml to 10^8 IU/ml HIV RNA for the RNA isolation from 1 ml of serum *(plasma)*.
1 IU = 0.58 copies of HIV RNA *(National Institute of Biological Standards and Control for WHO international standard for Human Immunodeficiency virus NIBSC Code: 97/656).*

Sensitivity:
Assay kit securely determines HIV RNA in concentration not less than 20 IU/ml for the RNA isolation from 1 ml of serum *(plasma)*.

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.

RealLine HIV quantitative Str-Format

6. STORAGE AND TRANSPORTATION

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

7. 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 label at the side of the box.

8. ADDITIONAL MATERIALS AND DEVICES REQUIRED BUT NOT SUPPLIED

- Real time PCR device RealLine Cycler (BIORON Diagnostics GmbH), iQ/iQ5 iCycler, CFX96 (Bio-Rad, USA), DT-96 (DNA-technology, Russia) or equivalent;
- RNA Extraction Kit: **RealLine Extraction 100** or **RealLine Extraction 1000**
- Tubes or plates and PCR foils, if the delivered format is not matching with the used system.
- Disposable gloves, powder-free;
- Pipettes (capacity 10-100 µl) with filters (aerosol barriers);
- Disposable DNAse/RNase-free tips with filters
- 0.2 ml microtube racks
- Razor or scalpel.

If an extraction kit from another supplier is used:

- RealLine Internal Control (VBC8881)
- Negative Control (negatively controlled plasma)

9. REAGENT AND SAMPLE PREPARATION

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

9.1 Sample preparation.

Prepare specimens for the assay with Extraction kit **RealLine Extraction 100** or **RealLine Extraction 1000** according to Extraction kit manual.

Note: For **quantification** process **three replica of Positive Control sample** and one Negative Control sample in each test run are necessary.

Attention: *In case of necessity to check the adequacy of analytical system, three replicas of both Calibration sample 1 and 2 (CS1 and CS2) should be used (see the Attachment 2)*, to confirm the correspondence between measured values and the passport. The analytical sensitivity can be monitored with the additional use of the WPC. The Ct-value should be less than 40.

Preparation of Control samples:
Add 1 ml of Recovery Solution for Control samples (RSC) into each vial with Positive Control (PC) sample and Weak Positive Control sample (WPC), mix gently, keep for 15 minutes, carefully mix once again.

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

9.2 Reagent preparation

Preparation of Ready Master Mix
Prior to use, warm reagents *(do not open!)* at room temperature (18 - 25) °C. Open the package, separate an appropriate number of reaction tubes with Ready Master Mix (RMM) using razor or scalpel.

Keep the tubes not used for the test in the original bag. Squeeze air out of the bag before closing the clip.

RealLine HIV quantitative Str-Format

10. PROCEDURE PROTOCOL

10.1 Place the tubes with processed specimens and controls to Magnetic Rack.

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

Attention! *Put marks on the lateral part of a reaction tube.*

10.3 Add **50 µl** of each processed specimen 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!**

10.4 Place reaction tubes into the thermal block of real time PCR device and program the settings respectively.
Program real time PCR device as follows:

Step 1:	45 °C	45 min	
Step 2:	95 °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			

- Collect real-time PCR data through the **FAM** channel for detection of amplification of IC cDNA.

- Collect real-time PCR data through the **ROX** channel for detection of amplification of HIV cDNA.

10.5 Start the PCR Run

11. DATA ANALYSIS

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

If **Ct** of IC for such samples 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.

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

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

11.3 In **Negative Control** sample the program should detect:

- **FAM** fluorescence signal increase and **Ct** value, and no significant **ROX** fluorescence increase should appear.
- If **Ct** value for **NC** in **ROX** channel is **less than 40**, this indicates the presence of contamination. All positive results in this test should be repeated from the RNA extraction stage. Negative samples of such test runs are considered reliable.

11.4 The sample is considered **positive** if **Ct** value in 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.

11.5 The test results are considered reliable only when Positive and Negative controls perform as expected.

Note: *Recommended setting for the Threshold is up to 20 % of the PC or the highest fluorescence signal, especially in not validated cyclers.*

RealLine HIV quantitative Str-Format

Summary of results:

	FAM IC	ROX HIV	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

HIV quantitation analysis.

- For quantitative analysis calculate the HIV RNA concentration in analyzed samples in accordance with *Attachment 1*.

Attention! *For RNA isolation from 100 µl of sample result should be multiplied by 10.*

- If calculated HIV 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 HIV RNA concentration in the sample *(in IU/ml).*

- Test results higher than 10^8 IU/ml are above the upper limit of quantitation and should be reported as **higher than 10^8 IU/ml.**

- Test results lower than 100 IU/ml are below the lower limit of quantitation and should be reported as HIV RNA detected, **lower than 100 IU/ml.**

- An analyzed sample is considered as negative if the result does not obtain a **Ct** value along the **ROX** channel or the **Ct** value exceed 40.

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.

ATTACHMENT 1: CALCULATION OF VIRAL RNA CONCENTRATION

Calculate the viral RNA concentration using the following equation:

$$c_{SAMPk} = c_{PC} * 2^{(Ct_{PC} - Ct_{SAMPk})} * 2^{(CT\ IC_k - Ct\ IC_{PC})},$$

Where:
k – sample number;
c_{PC} – PC concentration, specified in the passport of assay kit;
Ct_{PC} and $Ct\ IC_{PC}$ – **Ct** value of the PC sample **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%.**

ATTACHMENT 2: ANALYTICAL SYSTEM VALIDATION

Calculation of viral RNA concentration for PC using calibration graph.
For precise calculation of viral RNA concentration in analyzed samples, **analytical system** validation should be done by comparison of PC concentration, specified in the passport of Assay kit with PC concentration, calculated using calibration graph.
This validation is just necessary for the first time using the kit and to check the system regularly.

1. Prior use, warm reagents at room temperature (18 - 25) °C for 30 minutes. Prepare NC, PC and IC as recommended in the instruction.

2. Add 1 ml of Recovery Solution for Control samples (RSC) into a vial with Calibration sample 1 (CS1) and Calibration sample 2 (CS2). Mix gently, keep for 15 minutes, and carefully mix once again.

3. Prepare 10 2.0 ml tubes – one for NC, 3 repeats for each PC, CS1 and CS2.

4. Label one 2.0 ml tube for NC, and 3 2.0 ml tubes each for PC, CS1 and CS2

5. Add 30 µl of IC to each tube.

6. For NC, add to the tube, marked NC, 1 ml (100 µl) of Negative Control.

7. For PC, add to each of three tubes, marked PC, 970 (70) µl of Negative Control, and 30 µl of Positive Control.

8. For CS1 add to each of three tubes marked CS1 970 (70) µl of Negative Control, and 30 µl of Calibration sample 1.

9. For CS2 add to each of three tubes marked CS2 970 (70) µl of Negative Control, and 30 µl of Calibration sample 2.

10. Run the isolation of NA as recommended in the instruction.

RealLine HIV quantitative Str-Format

11. Run the PCR (or reversed PCR).

12. For each sample calculate correct Ct value, using the following equation:

$$\mathbf{z} = \boldsymbol{Ct_{Samp}} - (\boldsymbol{Ct\ IC_m} - \boldsymbol{Ct\ IC})$$

Calculate average z value for PC, CS1 and CS2 ($z_{PCm}, z_{CS1m}, z_{CS2m}$).
Samples with z, that differs from z_m more then 2, should be ignored. After screening, recalculate z_m for samples remained.

13. Calculate B – coefficient for calculating of specific viral NA, using following equation:

$$\boldsymbol{B} = \frac{\mathbf{log}(\boldsymbol{c_{CS1}}) - \mathbf{log}(\boldsymbol{c_{CS2}})}{\boldsymbol{z_{CS2m}} - \boldsymbol{z_{CS1m}}}$$

Where:
c_{CS1} and c_{CS2} – specific viral NA concentration in Calibration samples *(specified in the passport of Assay kit)*;
For amplification efficiency 100%: $B = \log 2 = 0{,}3$

14. Using received results, calculate concentration of specific viral RNA for Positive Control sample, using the following analytical equation:

$$\boldsymbol{c_{PC}} = \mathbf{10}^{\boldsymbol{XPC}}(\text{IU/ml})$$

Where:

$$XPC = \log(c_{CS1}) + B * (z_{CS1m} - z_{PCm})$$

z_{CS1m} – average Z value for Calibration Sample 1 repeats;
z_{PCm} – average Z value for Positive Control sample repeats;

15. If the calculated value of specific viral RNA concentration in PC differs no more than 2 from the value specified in the passport of a set, PC can be used as the sample of comparison for the further calculations of specific viral RNA concentration in investigated samples.

16. With the calculated Efficiency factor B it is possible to use this value in the formula for concentration calculation. (Attachment 1)

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.