Instructions for Use

RealLine EBV quantitative Str-Format

ASSAY KIT FOR THE QUANTITATIVE DETECTION OF EPSTEIN-BARR VIRUS DNA (EBV) BY REAL-TIME PCR METHOD

In vitro Diagnostics

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

CE

RealLine EBV quantitative (Str-Format)	**VBD2198**	**96 Tests**
valid from	**October 2019**	

BIORON Diagnostics GmbH
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Explanation of symbols used in labeling

IVD	*In vitro* diagnostic medical device
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

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1. INTENDED USE

Clinical information:

The Epstein-Barr virus (EBV; also Human Herpesvirus 4, HHV-4) is a human pathogen, enveloped, double-stranded DNA virus. Infection with the virus happens usually during childhood, without any symptoms. In adolescent or adults it comes to an outbreak of Pfeiffer glandular fever (infectious mononucleosis) in 30-60% of cases. From the 40th about 98% of people are infected with EBV. According to both asymptomatic and symptomatic after infection, the virus persists in the body for life and can be reactivated as all herpes viruses. Usually a reactivation is not noticed by the host and quickly antagonized by the immune system. In case of immunosuppression (eg, HIV or transplant recipients), the virus can multiply uncontrollably and contribute to the emergence of various rare cancers.

RealLine EBV quantitative (Str-format) assay kit is designed to detect Epstein-Barr virus DNA isolated from clinical specimens using extraction kits:
RealLine DNA-Express (REF VBC8899)
RealLine DNA-Extraction 2 (REF VBC8897)
RealLine DNA-Extraction 3 (REF VBC8889)
RealLine Extraction 100 (REF VBC8896)

RealLine EBV assay kit is intended for the detection of Epstein-Barr virus DNA in clinical specimens (whole blood, serum, plasma, cerebrospinal fluid, oropharyngeal swabs, saliva, biopsy materials, bronchopulmonary lavage) and for quantitative determination of EBV DNA in whole blood and serum (plasma) using the method of real-time polymerase chain reaction (PCR) with fluorescence detection of amplified product.

When extracting EBV DNA from blood serum (plasma) with "RealLine Extraction 100" kit and from whole blood using "RealLine Extraction 100" and “RealLine Hemolytic” kits a quantitative determination of EBV DNA in the specimen is possible.

For a quantitative determination of EBV DNA from blood serum (plasma, use the extraction kits: **RealLine DNA-Extraction 2 or RealLine Extraction 100** and follow the instructions below.

The results of PCR analysis are taken into account in complex diagnostics of disease.

The **Str-Format Kit** contains 96 tubes (0.2 ml) in strips with lyophilized 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 96 tests, including control samples and the positive control sample.

The kit is validated for use with iQ™ iCycler, iQ™5 iCycler, CFX96™ (Bio-Rad, USA) DT-96 (DNA-Technology, Russia) and RealLine Cycler (BIORON Diagnostics GmbH).

The use of:

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

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

Universal Positive Control sample (UPC) certified according the reference standard 1st WHO International Standard for Epstein-Barr virus (EBV)" (NIBSC code 09/260)	1 vial, 1 ml;
Ready Master Mix RMM for PCR (MM), lyophilized	96 test-tubes
The kit is additionally supplied with optical-transparent PCR-film	
Passport with concentration of EBV in the UPC (Universal positive control) at a sticker in the upper lid of the box	

3. PRINCIPLE OF THE METHOD

The 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 dual-labeled DNA-probe, which specifically binds to the target region of pathogen DNA. Fluorescent signal increases due to the fluorescent dye and quencher separating by Taq DNA-polymerase exonuclease activity during amplification. PCR process consists of repeated cycles: temperature denaturation of DNA, primer annealing and complementary chain synthesis.

Threshold cycle value – Ct – is the cycle number at which the fluorescence generated within a reaction crosses the fluorescence threshold, a fluorescent signal rises significantly above the background fluorescence. Ct depends on initial quantity of pathogen DNA template.
The use of **Internal Control (IC)** prevents generation of false negative results associated with possible loss of DNA template during specimen preparation. IC indicates if PCR inhibitors occur in the reaction mixture. IC template should be added in each single sample (including control samples) prior to DNA extraction procedure. The amplification and detection of IC does not influence the sensitivity or specificity of the target DNA PCR.

Note: IC is a component of the NA extraction kits of RealLine 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

The range of detectable concentrations (linearity range): 500 to 10^8 IU EBV DNA/ml.

4.1. **Specificity** of the EBV DNA detection is determined using the Standard Reference Panel of negative sera, SRP NCS, as a percentage of the samples determined by the kit as negative. Specificity equals 100%.

4.2. **Sensitivity** is determined on five samples containing 40 IU of EBV DNA in the sample, prepared from Standard Reference Sample, SRS EBV DNA, as a percentage of the samples, determined by the kit as positive. Sensitivity equals 100%.

4.3. The **coefficient of variation** (CV in %) is calculated for the logarithm values of the EBV DNA concentrations in six SRS samples. The coefficient of variation is not more than 10%.

4.4. The **"linearity"** test characterizes the coincidence (in %) of the measured value and the prescribed value (calculated with allowance for the dilution factor) of the logarithm of the EBV DNA concentration in a sample prepared by dilution from SRS. The "linearity" is in the range of 90-110%.

4.5. **Diagnostic sensitivity** of the EBV DNA detection: clinical tests conducted on 105 positive samples from 80 patients diagnosed with infectious mononucleosis and HIV-infected patients showed 100% sensitivity (interval 96.3% -100%, with a confidence level of 90%).

4.6. **Diagnostic specificity** of the EBV DNA detection: clinical trials conducted on 96 negative samples from nominally healthy blood donors and HIV-infected patients showed 100% specificity (interval 96.9% -100%, with a confidence level of 90%).

Analysis by the CE-marked reference kit showed full match of results.

5. LIMITATIONS

- This assay must not be used with the clinical specimen directly. Appropriate nucleic acids extraction methods have to be conducted prior to using this assay.
- The presence of PCR inhibitors (e.g. heparin) may cause underquantification, false negative or invalid results.
- When monitoring a patient the same extraction method must be used in all determinations. Otherwise, results may not be relative.
- Results should be interpreted with consideration of clinical and laboratory findings.
- Reliable results depend on adequate specimen sampling.
- Negative results indicate lack of detectable DNA in the examined sample type, but do not exclude the infection or disease.
- Positive results indicate active or asymptomatic infection; viral load and a clinical history and symptoms should be taken into account.
- Potential mutations within the target regions of the EBV genome covered by the primers and/or probes used in the kit may result in underquantification and/or failure to detect the presence of the pathogens.

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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 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 p.1
- DNA-Extraction Kit, RealLine DNA-Extraction 2 or see p.1 Extractions Kits with Internal control reagent;
- Internal Control reagent (VBC8881) and Negative Control Sample, if the kit is used with the extraction kits of other supplier;
- Safety laminar cabinet;
- Refrigerator;
- Half-automatic variable-volume single-channel pipettes;
- Disposable medical non-sterile powder-free gloves;
- Disposable pipette tips with aerosol barrier;
- Biohazard waste container
- Scalpel or scissors

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.

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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.

The assay is performed on extracted DNA specimens obtained from the clinical material using one of the DNA extraction kits listed in p.1.2, according to the Instruction Manual. If an extraction kit with magnetic particles is used, keep the tubes with extracted DNA in the magnetic rack.

In case of quantitative determination, the DNA extraction is conducted from 100 µl of blood serum or 100 µl of plasma specimen (*with EDTA as anticoagulant*) using **RealLine Extraction 100** kits. For analysis of whole blood the specimen volume should be 250 µl, each specimen must undergo the preliminary treatment with **RealLine Hemolytic** kit. Specimens are then ready to undergo the DNA extraction procedure with **RealLine Extraction 100** kit. Each group of specimens should include 3 PC and 1 NC. Elution volume is 200 µl.

Store the extracted DNA at (2 – 8) °C for no more than 24 hours.
After initial opening, store PC at (2 – 8) °C for no more than 1 month or in 50 µl aliquots at minus (18 – 24) °C during 3 months.

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9. PROCEDURE

9.1. Preparation of the Kit Components.

Prior the test, take the kit out of the refrigerator and keep the **Ready Master Mix for PCR (RMM)** and **PC** closed in the package at (18 – 25) °C for at least 30 min. Open the package and cut the necessary number of tubes with RMM (*including prepared specimens and control samples: 1 PC and 1 NC for qualitative detection; 3 PC and 1 NC for quantitative assay*) with the razor or scalpel.

Cut the tubes together with the covering film. Put the remaining tubes immediately back into the foil pouch, squeeze the air out and tightly close with the clip.
After initial opening of the package, store RMM at (2 - 8) °C for no more than 3 months.

9.2. Label the tubes with RMM for each specimen and control.
Attention! *Labels should be placed on the lateral side of the tubes.*

9.3. Add **50 µl** of corresponding isolated DNA solution to each tube using a separate pipette tip with filter. Do not touch the pellet! Tightly close the tubes with caps or seal with the PCR transparent film.

9.4. Place the tubes into the Real Time PCR system.

9.5. Program Real Time PCR system as follows:

Stage 1:	50°C	2min	
Stage 2:	95°C	2min	
Stage 3:	94°C	10 sec	50 cycles
	60°C*	20 sec	
* Measure the fluorescence at 60°C			

9.6. 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 EBV DNA.

9.7. 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 and run the program.

10. DATA ANALYSIS AND INTERPRETATION

10.1 For **PC** the program should:

- detect an increase in **IC DNA** amplification signal (**FAM** channel) and determine IC Ct;
- detect an increase in **EBV DNA** amplification signal (**ROX** channel) and determine PC Ct.

10.2 For **NC**, the program should detect the increase in the amplification signal of **IC DNA** (channel **FAM**) and determine IC Ct. No **ROX** fluorescence increase should appear (no EBV DNA amplification).

10.3 For each specimen the program should detect the increase in the amplification signal of **IC** DNA (channel **FAM**) and determine IC Ct.

10.4 Calculate $(IC\ Ct)_{av}$ as an average IC Ct of all specimens (including PC and NC). IC Ct values that differ by more than 2 from the $(IC\ Ct)_{av}$ should be ignored. Recalculate the $(IC\ Ct)_{av}$ for the remaining values.

10.5 The specimen is considered **positive**, i.e. containing **EBV DNA,** if Ct value via ROX channel for this specimen is **less than or equals to 40.**

10.6 The specimen is considered **negative**, if Ct value through **ROX** channel for this specimen is **above 40 or is not determined**. If IC Ct value for such specimen differs from $(IC\ Ct)_{av}$ value by more than 2, the result is regarded as **equivocal**. A repeated analysis of the specimen, starting from the DNA extraction step is required.

10.7 If the **Ct** value for **NC** through the **ROX** channel **is less than or equals to 40,** it indicates the presence of contamination. In this case, all positive results of this individual PCR run are considered **equivocal**. Actions are required to identify and eliminate the source of contamination, and repeat the analysis of all specimens of this run that were identified as positive. Specimens that showed negative results in this run should be considered negative.

10.8 Quantitative analysis of results.
For quantitative analysis calculate **EBV DNA** concentration in analyzed specimens according to **Annex II.**

- If the calculated value of the **EBV DNA** concentration in the specimens is in the range from **500 to 10^8 IU of EBV DNA/ml**, the result is determined **positive** and the obtained concentration of **EBV DNA** (IU/ml) is indicated.
- If the calculated value of the **EBV DNA** concentration is greater than **10^8 IU,** the result should be interpreted **positive** with EBV DNA concentration **greater than 10^8 IU/ml**.
- If the calculated value of the **EBV DNA** concentration is less than **500 IU/ml**, the result should be interpreted **positive** with EBV DNA concentration **less than 500 IU/ml**.

10.9 The specimen is considered **negative** (not containing EBV DNA), if the value of Ct through the **ROX** channel for this specimens is **above 40 or is not determined.**

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11. STORAGE AND TRANSPORTATION

- Store the assay kit at (2 - 8) °C in the manufacturer's packing.
- Transport at (2 - 8) °C . Transportation at 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 *or in 50 µl aliquots at minus (18 – 24) °C during 3 months.*
  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.

ANNEX II: CALCULATION OF EBV DNA CONCENTRATION

Quantitative analysis of EBV DNA should be performed as follows:

1. For PC with the Ct value lying in the range indicated in the insert to the kits of this lot, calculate the adjusted EBV Ct values by applying a correction for extraction efficiency, controlled by IC Ct, according to the formula:

$$Z_{PC} = PC\ Ct + [(IC\ Ct)_{av} - IC\ Ct], \text{ where:}$$

Z_{PC} is the adjusted value of EBV Ct for PC sample;
$(IC\ Ct)_{av}$ is the average IC Ct calculated over all specimens (see the Instruction Manual);
PC Ct and IC Ct are the Ct values of this PC sample through **ROX** and **FAM** channels, respectively.

2. Calculate the average value of the adjusted Ct for PC (Z_{PCav}).

Discard Z_{PC} that differs from the Z_{PCav} value by more than 2 and calculate Z_{PCav} for the remaining specimens.

3. Calculate the quantity of EBV DNA in each specimen (Q_k) according to the formula:

$$Q_k = C_{PC} \times 2^{Xk}\ (IU),$$

$$\text{where: } X_k = Z_{PCav} - Z_k;$$

C_{PC} is the concentration of EBV DNA in PC indicated in the inlet to the kit, IU/ml;
k is the specimen number;
Z_k is the adjusted Ct value of the specimen calculated as follows:

$Z_k = EBV\ Ct_k + [(IC\ Ct)_{av} - (IC\ Ct)_k]$.
4. Calculate the concentration of EBV DNA C_k (in IU/ml) as follows:
$C_k = 10 \times Q_k$, when extracting from 100 µl of specimen;
$C_k = 4 \times Q_k$, when extracting from 250 µl of specimen.
Thus, EBV DNA concentration in the specimen is calculated and expressed in IU/ml.

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

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SPACE FOR YOUR NOTES:

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