

Instructions for Use

RealLine HHV-6 Fla-Format

**ASSAY KIT FOR THE QUALITATIVE DETECTION OF HUMAN HERPES VIRUS TYPE 6
(HHV-6) DNA BY REAL-TIME PCR METHOD**










In vitro Diagnostics



RealLine HHV-6 (Fla-format)	VBD2151	50 Tests
valid from:	September 2019	

RealLine HHV-6 Fla-Format

Explanation of symbols used in labeling

	<i>In vitro</i> diagnostic medical device
	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@bioron.de

Trademarks:

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

RealLine HHV-6 Fla-Format

Table of content

1. INTENDED USE AND INTRODUCTION	4
2. KIT CONTENTS	5
3. PRINCIPLE OF THE METHOD	5
4. SPECIFICATIONS	6
5. PRODUCT USE LIMITATIONS	6
6. WARNING AND PRECAUTIONS	7
7. ADDITIONAL MATERIALS AND DEVICES REQUIRED BUT NOT SUPPLIED	7
8. PREPARATION OF THE SPECIMEN	8
9. PROCEDURE	8
10. DATA ANALYSIS AND INTERPRETATION	10
11. STORAGE AND TRANSPORTATION	11
ANNEX I: Settings for RealLine Cyclers and DT96:	11
ANNEX II: Programming the device and analysis of results using Rotor-Gene cyclers:	11

RealLine HHV-6 Fla-Format

ASSAY KIT FOR THE DETECTION OF HUMAN HERPES VIRUS TYPE 6 DNA BY REAL-TIME PCR METHOD

In vitro Diagnostics

1. INTENDED USE AND INTRODUCTION

Clinical Information:

HHV-6 infections can asymptotically occur early in life with lifelong persistence. Primary infection with HHV-6 causes roseola infantum, a common childhood disease that resolves spontaneously. The virus remains latent in lymphocytes and monocytes and persists at low levels in cells and tissues. It can reactivate at a later date, and is believed to contribute to diseases of the bone marrow and/or central nervous system in some patients, including mononucleosis like illness with fever, lymphadenopathy, hepatitis, fatal encephalitis, chronic fatigue syndrome, and possibly multiple sclerosis. Transmission of HHV-6 is believed to occur as a result of exposure to saliva.

RealLine HHV-6 assay kit is designed to detect human herpes virus type 6 DNA isolated from clinical specimens using extraction kits:

RealLine DNA-Express (REF VBC8899)

RealLine DNA-Extraction 3 (REF VBC8889)

RealLine Extraction 100 (REF VBC8896)

RealLine HHV-6 kit is designed for the analysis of clinical materials: oropharyngeal swabs, cerebrospinal fluid, urine, biopsy material, swabs of epithelial cells, semen, prostatic fluid, serum, plasma, whole blood, bronchoalveolar lavage, using the method of real-time polymerase chain reaction (PCR) with fluorescence detection of amplified product.

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

The **Fla-format** Kit contains 5 vials with the lyophilized Mastermix, each vial with 10 reactions, for volume of 50 µl per reaction. The kit contains reagents required for 50 tests, including the positive control samples.

The kit is designed for use with block cyclers iQ™ iCycler, iQ5™ iCycler, CFX96™ (Bio-Rad, USA), DT96 (DNA-Technology, Russia) RealLine Cycler (BIORON Diagnostics GmbH); and rotor type cyclers Rotor-Gene® 3000, 6000 and Q (Qiagen, Germany).

For the Eco48™ Realtime PCR System (PCRmax, UK) RealLine Fla-format kits can be recommended. The practice with this cycler to use 10 µl of the diluted Mastermix and 10 µl of extracted DNA, was validated. The protocol for using and cycling can be provided.

RealLine HHV-6 Fla-Format

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

Universal Positive Control Sample (PC) -	1 vial, 1 ml
Master Mix (MM) , lyophilized	5 tubes, 10 tests each
Recovery Solution (RS)	2 vials, 2 ml each

3. PRINCIPLE OF THE METHOD

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 DNA-probe that specifically binds to the target region of pathogens DNA. Fluorescence signal increases due to the separation of fluorescence dye and quencher by Taq DNA polymerase exonuclease activity during amplification. PCR consists of repeated cycles: temperature denaturation of DNA, 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 DNA hybridization probe that is specific for the given DNA sequence: it binds to one of the DNA strands in the course of reaction and provides additional specificity of the method. A DNA 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 DNA polymerase, the quencher and the dye become separated, thus increasing the fluorescence signal due to accumulation of the reaction product. The detected fluorescence intensity depends on initial quantity of pathogens DNA template in the sample.

The use of **Internal Control sample (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 should be added to each sample (including control samples) prior to the DNA extraction procedure. Amplification and detection of IC influence neither sensitivity nor specificity of the target DNA PCR.

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

RealLine HHV-6 Fla-Format

4. SPECIFICATIONS

- 4.1. Sensitivity** is determined on five samples, containing 100 copies of HHV-6 DNA per sample prepared from HHV-6 DNA Standard Reference Sample (SRS), as percentage of samples detected by the kit as positive. Sensitivity equals 100 %.
- 4.2. Specificity** of HHV-6 DNA detection is determined using the Standard Reference Panel of negative DNA extracts as percentage of samples detected by the kit as negative. Specificity equals 100 %.

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

5. PRODUCT USE LIMITATIONS

- This assay must not be used on 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 false negative or invalid results.
- When monitoring a patient the same extraction method must be used in all determinations. Otherwise, results may not be comparable.
- The kit is designed for use in patients with a clinical history and/or symptoms consistent with HHV-6 infections. The kit may be used for screening purposes.
- Diagnostic sensitivity of the kit may vary depending on the pathogen prevalence and characteristics of the enrolled cohort.
- Reliable results depend on adequate specimen sampling.
- Positive results indicate active or asymptomatic infection; clinical history and symptoms should be taken into account.
- Negative results indicate lack of detectable DNA but do not exclude the infection or disease.
- Potential mutations within the target regions of the HHV-6 genome covered by the primers and/or probes used in the kit may result in failure to detect the presence of the pathogens.
- The kit is not intended to replace culture and other methods (e.g., cervical exam) for diagnosis of infections.

RealLine HHV-6 Fla-Format

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-Express, RealLine DNA-Extraction 3 or RealLine Extraction 100**
- Internal Control reagent (VBC8881) and Negative Control Sample, if the kit is used with the extraction kits of other supplier.
- Plates or Tubes suitable for the used device with caps or a sealing foil for PCR
- 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.

RealLine HHV-6 Fla-Format

8. PREPARATION OF THE SPECIMEN

The assay is performed on extracted DNA samples obtained from the clinical material using one of the DNA extraction kits listed in p.1, according to the Instruction Manual to the kit.

*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 p1., 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".

If samples of isolated DNA were stored frozen prior the assay, thaw them and keep at least 30 minutes at a temperature of (18 – 25) °C.

The isolated DNA can be stored at (2 – 8) °C for 24 hours.

After initial opening shelf life of Positive Control sample is 1 month at (2 – 8) °C or for 50 µl aliquots 3 month at (-18 ... -60) °C

9. PROCEDURE

9.1. Preparation of the kit components

Prior to the test, take the kit out of the refrigerator, open the package, take the necessary number of tubes with the **Master Mix for PCR (MM)**, taking into account prepared specimens and control samples. 1 NC and 1 PC for each test is required. Keep the tubes at (18 – 25) °C for at least 30 min.

Put the remaining tubes immediately back into the foil pouch, squeeze the air out and tightly close with the clip.

Attention! Each tube is intended for 10 tests.

After initial opening, store MM at (2 – 8) °C for no more than 3 months.

To prepare diluted Master Mix, add **300 µl of Recovery Soltion RS** to each tube with MM. Mix gently, keep at (18 - 25) °C during 15 min, and mix thoroughly one more time. Collect the tube contents by brief centrifugation.

Store diluted MM at (2 – 8) °C for no more than 7 days.

After initial opening, store RS at (2 – 8) °C for no more than 3 months.

RealLine HHV-6 Fla-Format

9.2. Prepare an appropriate number of 0.2 ml tubes or a plate for PCR. Label each tube for each specimen and control.

Attention! Labels should be placed on the caps of tubes for rotor-type cyclers, for block-type cyclers labels should be placed on the lateral side of the tubes.

9.3. Add **25 µl** of prepared Master Mix to each 0.2 ml tube.

9.4. Add **25 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 or plate.

9.5. Place the tubes into the real-time PCR system.

9.6. Program real time PCR system.

For Rotor-Gene® 3000 (6000, Q):

Step 1:	50°C	2 min	50 cycles
Step 2:	95°C	2 min	
Step 3:	94°C	10 sec	
	60°C*	40 sec	

* Measure the fluorescence at 60°C.

For RealLine Cycler, iQ™ iCycler, iQ™5 iCycler, CFX96™, DT-96:

Step 1:	50°C	2min	50 cycles
Step 2:	95°C	2min	
Step 3:	94°C	10 sec	
	60°C*	20 sec	

* Measure the fluorescence at 60°C

9.7. Select the amplification detection channels:

- Collect data through **FAM** channel (iQ5 iCycler, CFX96, RealLine Cyclers, DT-96, Rotor-Gene 3000) or **Green** channel (Rotor-Gene 6000, Rotor-Gene Q) for the detection of amplification signal of **IC DNA**;
- Collect data through **ROX** channel (iQ5 iCycler, CFX96, RealLine Cyclers, DT-96, Rotor-Gene 3000), or **Orange** channel (Rotor-Gene 6000, Rotor-Gene Q) for the detection of amplification signal of **HHV-6 DNA**;

9.8. Program the position of the tubes with the specimens, PC and NC according to the Instruction Manual for the cycler in use.

9.9. Run the program.

RealLine HHV-6 Fla-Format

10. DATA ANALYSIS AND INTERPRETATION

- 10.1** The program should detect in **Positive Control** sample:
- increase of the **IC DNA** amplification signal along channel **FAM** (Green) and determine the threshold cycle, IC Ct;
 - increase of the **HHV-6 DNA** amplification signal along channel **ROX** (Orange) and determine the Ct value;
- 10.2** For **NC** the program should detect the increase of the amplification signal of IC DNA along channel **FAM** (*Green*) and determine the threshold cycle, IC **Ct**. No significant **ROX** (*Orange*) fluorescent increase should appear (*no HHV-6 DNA amplification*).
- 10.3** For each sample the program should detect the increase of the amplification signal of IC DNA along channel **FAM** (*Green*) and determine IC **Ct**.
- 10.4** Calculate (IC **Ct**)_{av} as an average IC **Ct** of all analyzed samples (including PC and NC). IC **Ct** values that differ by more than 2 cycles from the (IC **Ct**)_{av} should be ignored. Recalculate the (IC **Ct**)_{av} for the remaining values after the screening.
- 10.5** The sample is considered **negative** (not containing HHV-6 DNA), if **Ct** value via **ROX** (*Orange*) channels for this sample is **above 40** or is not determined.
If IC **Ct** value for such sample differs from the (IC **Ct**)_{av} value by more than 2 cycles, the result is regarded as equivocal. A repeated analysis of the sample, starting with the DNA isolation step is necessary.
- 10.6** The sample is considered **positive** (containing *HHV-6 DNA*), when **Ct** value via **ROX** (*Orange*) channel for this sample is **less than or equals to 40**.
- 10.7** If Ct value for NC through **ROX/Orange** channels is less than or equal to 40, this 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 determined as positive. Specimens that showed negative results in this run should be considered negative.

RealLine HHV-6 Fla-Format

11. STORAGE AND TRANSPORTATION

- Store the assay kit at (2 - 8) °C in the manufacturer's packing.
 - Transport the kit 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 - 60) °C for up to 3 months.
 - Ready Master Mix (MM): unused MM at (2 - 8) °C for no more than 3 months
 - Diluted MM: at (2 - 8) °C for 7 days in a dark surrounding
 - Recovery Solution: at (2 - 8) °C for 3 months.

ANNEX I: Settings for RealLine Cyclers 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: 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).

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

Step 1:	50°C	2min	50 cycles
Step 2:	95°C	2min	
Step 3:	94°C	10 sec	
	60°C*	40 sec	

* Measure the fluorescence at 60°C

- 6) Then temperature profile is set, click **OK** button.
- 7) 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**.

RealLine HHV-6 Fla-Format

- 8) Tick off **Perform Calibration Before 1st Acquisition**. Click **Close** button.
- 9) Click **Next** button, start the amplification process by clicking **Start Run** button.
- 10) 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.
- 11) Save reaction result file with Rotor-Gene Run File *.rex extension.
- 12) Record the positions of the controls and specimens according to the instruction manual of the operating device. Click **Start run** button.

Results of IC DNA amplification

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

Results of HHV-6 DNA amplification

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

Technical support: techsupport@bioron.de



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