Instructions for Use

RealLine HPV 6 / 11
Str-Format

QUALITATIVE ASSAY KIT FOR THE DIFFERENTIAL DETERMINATION OF DNA FROM HUMAN PAPILLOMAVIRUS TYPES 6 AND 11 BY REAL TIME PCR METHOD

In-vitro Diagnostics

CE

RealLine HPV 6 / 11 (Fla-Format)	**VBD8475**	**96 Tests**
valid from	**September 2023**	

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

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Explanation of symbols used in labelling

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

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

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1. INTENDED USE

Clinical information:
Human Papilloma Viruses HPV are DNA-Viruses, and more than 100 different types are known at that moment. While the majority of HPVs cause no symptoms, some can cause low severe symptoms like warts and a few types are known to cause cancer. HPV types that are more likely to lead to the development of cancer are referred as High-Carcinogenic-Risk types HPV. The HPV Types 6 and 11 are known as Low-Carcinogenic-Risk-Types and cause genital warts.

The extraction of DNA from clinical materials can be performed using the extraction kits:

- **RealLine DNA-Express (REF VBC8899-R)**
- **RealLine Extraction 100 (REF VBC8896-R)**
- **INSTANT Virus RNA/DNA Kit – IPC16 (Roboscreen GmbH, Germany)**
- **INSTANT Virus RNA/DNA Kit – FX (Roboscreen GmbH, Germany)**
- **VeriLab UMag (Laboveritas SIA)**

When using DNA extraction kits of other manufacturers, it necessary to use Internal Control sample (IC) (VBC8881, BIORON Diagnostics).

RealLine HPV 6/11 (Str-format) assay kit is intended for the differential detection of human papillomavirus (HPV) types 6 and 11 DNA in clinical specimens (cervical swabs) using real-time polymerase chain reaction (PCR) with fluorescence detection.
The assay is based on real-time polymerase chain reaction (PCR) method with fluorescent detection of amplified product.

The **Str-Format Kit** contains 96 tubes (0.2 ml) in strips with lyophilized master mix. 50 µl of extracted DNA have to be pipetted into the tube and the ready master mix 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™5 iCycler (Bio-Rad, USA. The kit is compatible with real-time PCR systems such as iQ™ iCycler, CFX™96 (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 another 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)	1 vial, 1 ml
Ready Master Mix (RMM), lyophilized	96 test-tubes 12 strips x 8 tubes
The kit is additionally supplied with optical-quality PCR-film	

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

4.1. Sensitivity:

Sensitivity control was performed on 5 samples containing 100 DNA copies per sample types HPV 6 and 11, prepared from Standard Reference Samples containing DNA of HPV types 6 and 11, (HPV 6 DNA SRS; HPV 11 DNA SRS).
The sensitivity equals 100 %.

4.2. Specificity:

Specificity of HPV 6 and 11 DNA detection was determined using 5 negative DNA-extracts samples not containing DNA of STD agents or herpes virus infections, including HPV types 6 and 11 DNA, and containing IC DNA.
Specificity of HPV 6/11 DNA detection equals 100 %.

4.3. Diagnostic evaluation:

Diagnostic evaluation was performed on 50 clinical samples:
10 clinical samples obtained from healthy donors;
8 clinical samples obtained from patients with STD symptoms;
3 clinical samples obtained from patients with diagnosed type of HPV 16, 18, 35;
18 clinical samples obtained from patients with cytological signs of HPV infection.

All samples were analyzed with the **RealLine HPV 6 / 11** assay kit (BIORON Diagnostics) and the CE-marked reference kit.
Results obtained show total coincidence between the RealLine DNA HPV 6/11 assay kit and the CE-marked reference kit – 100 % sensitivity and specificity according to the reference kit.

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 HPV Low carcinogenic Risk Types 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 HPV Low carcinogenic Risk Types 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.

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 paragraph 1
- **DNA-Extraction Kits listed in p.1**
- **Internal Control reagent** (VBC8881) and Negative Control Sample , if the kit is used with the extraction kits of other supplier.
- laminar safety 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 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 kits 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.

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.

Store the extracted DNA at (2–8) °C for no more than 24 hours.
After initial opening shelf life of Positive Control sample is 1 month at (2 – 8) °C or for 50 µl aliquots 3 months at (-18 - -60) °C

9. PROCEDURE

9.1. Preparation of the reagents.

Prior the test, take the kit out of the refrigerator and keep the **Ready Master Mix (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 and 1 PC*) with the scissors. 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.

After initial opening the shelf life of RMM at (2 – 8) °C is 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. Carefully remove the covering film so that RMM remains in the tube.

9.4. 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.

Note: For more efficient mixing, it is recommended to shake the PCR plate on the plate shaker at 1,200–1,800 rpm for at least 1 min.

Start the PCR no later than 30 min after the DNA addition.

Attention! *The optical film should remain clean (free of any labels or marks).*

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

Program Real Time PCR system as follows:

Step 1:	50°C	2min	
Step 2:	95°C	2min	
Step 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 **HEX** channel for detection of amplification of **HPV 6 DNA**.
- Collect Real Time PCR data through the **ROX** channel for detection of amplification of **HPV 11 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.

9.8. Run the program.

10. DATA ANALYSIS AND INTERPRETATION

10.1 For **PC** the program should detect an increase of the **IC DNA** amplification signal in **FAM** channel and determine the threshold cycle **IC Ct**; an increase of the **HPV 6** DNA amplification signal in **HEX** channel and determine the **Ct** value and an increase of the **HPV 11** DNA amplification signal in **ROX** channel and determine the **Ct** value;

10.2 For **NC** the program should detect the increase of the amplification signal of IC DNA (channel **FAM**) and determine the threshold cycle, IC **Ct**. No significant **HEX** and **ROX** fluorescent increase should appear (*no HPV 6 and 11 DNA amplification*).

10.3 For each sample the program should detect the increase of 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 analyzed samples (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 after the screening.

10.5 The sample is considered **negative** (not containing HPV 6 or HPV 11 DNA), if **Ct** value via **HEX** and **ROX** 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, 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 *HPV 11 DNA*) when **Ct** value via **ROX** channel for this sample is **less than or equals to 40.**

The sample is considered **positive** (containing *HPV 6 DNA*) when **Ct** value via **HEX** channel for this sample is **less than or equals to 40**

10.7 If the Ct value for **NC** in the **HEX/JOE/Yellow** and/or **ROX/Orange** channels is less **than or equal to 40**, this is indicative of the presence of **contamination** in the system. In this case, the positive results of this individual PCR test run are considered invalid. Take measures to detect and eliminate the source of contamination and repeat the assay for all the samples of this test run which had a positive result. The samples for which the test has yielded negative results should be considered negative.

11. STORAGE AND TRANSPORTATION

- Store the assay kit at (2 - 8) °C in the manufacturer's packing for the entire shelf life.
- The shelf life of the kit is 12 months from the manufacture date.
- 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 for 50 µl aliquots 3 months at (-18 - -60) °C
  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.

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