Instructions for Use

RealLine HPV 16 / 18
Str-Format

QUALITATIVE ASSAY KIT FOR THE DIFFERENTIAL DETERMINATION OF HUMAN PAPILLOMAVIRUS TYPE 16 AND 18 DNA BY REAL TIME PCR METHOD

In vitro Diagnostics

CE

RealLine HPV 16 / 18 (Str-Format)	**VBD8473**	**96 Tests**
valid from	**September 2023**	

BIORON Diagnostics GmbH
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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

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1. INTENDED USE

Clinical information:
Human Papilloma Viruses HPV are DNA-Viruses and more than 100 different types are known. While the majority of HPVs cause no symptoms, some can cause low severe symptoms like warts and a few are known to cause cancer. HPV types that are more likely to lead to the development of cancer are referred as high-risk types HPV. High-risk HPV-types are known to cause the vast majority of cervical cancers which cause death in women with an annual incidence of around half a million and a mortality of almost 50 %. HPV 16 and 18 types are the most prevalent high carcinogenic risk types.,

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 16 / 18 assay kit is intended for differential detection of DNA of human papillomavirus (HPV) types 16 and 18 DNA in clinical specimens (epithelial cell swabs) 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 **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™5 iCycler (Bio-Rad, USA, 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 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
Ready Master Mix (RMM), lyophilized	96 test-tubes (12 strips x 8 tubes);

The kit also includes PCR optical quality film – 1.5 sheets.

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-labelled 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** of HPV 16 DNA detection is determined on five samples prepared from HPV 16 DNA Standard Reference Sample with HPV 16 DNA content 100 copies per sample - 100%.

Sensitivity of HPV 18 DNA detection is determined on five samples prepared from HPV 18 DNA Standard Reference Sample with HPV 18 DNA content 100 copies per sample - 100%.

4.2. **Specificity** of HPV 16 DNA detection is determined on five samples prepared from HPV 18 DNA Standard Reference Sample with HPV 18 DNA content 100 copies per sample - 100%.

Specificity of HPV 18 DNA detection is determined on five samples prepared from HPV 16 DNA Standard Reference Sample with HPV 16 DNA content 100 copies per sample - 100%.

4.3. **Diagnostic sensitivity** of detection of DNA of human papillomavirus types 16 and 18: clinical trials conducted on 92 positive samples showed 100% sensitivity (interval 96.8% -100%, with a confidence level of 90%).

4.4. **Diagnostic specificity** of detection of DNA of human papillomavirus types 16 and 18: clinical trials conducted on 94 negative samples showed 100% specificity (interval 96.9% -100%, with a confidence level of 90%).

Analysis of similar samples by CE-marked reference kit confirms the results obtained in all cases.

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 Human Papilloma Virus 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 Human Papilloma Virus 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.

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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 rea-gents.
- 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 box.

7. ADDITIONAL MATERIALS AND DEVICES REQUIRED BUT NOT SUPPLIED

- real time PCR system, like described in p.1
- DNA-Extraction Kit: 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;
- 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.
- scalpel or pair of scissors

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. If an extraction kit with magnetic particles is used, keep the tubes with extracted NA in a magnetic rack.

*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

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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.

Store the extracted DNA at (2–8) °C for no more than 24 hours.
After initial opening 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. 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. Tightly close the tubes with caps or seal with the PCR transparent film.

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

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.

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

9.6. 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

9.7. 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 16 DNA**.
- Collect real-time PCR data through the **ROX** channel for detection of amplification of **HPV 18 DNA**.

9.8. 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.

9.9. Run the program.

10. DATA ANALYSIS AND INTERPRETATION

10.1 For PC the program should detect:

- detect an increase in the **IC DNA** amplification signal (**FAM** channel) and determine the threshold cycle, IC Ct;
- detect an increase in the **HPV16 DNA** amplification signal (**HEX** channel) and determine the Ct value;
- detect an increase in the **HPV18 DNA** amplification signal (**ROX** channel) and determine the Ct value.

10.2 For **NC** the program should detect the increase in the amplification signal of **IC DNA** (FAM channel) and determine IC Ct. No significant **HEX** and **ROX** fluorescent increase should appear (no HPV16 and 18 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 $(IC\ Ct)_{av}$ should be ignored. Recalculate $(IC\ Ct)_{av}$ for the remaining values.

10.5 The specimen is considered **negative** (not containing **HPV16 or HPV18 DNA**), if Ct through **HEX** and **ROX** channels for this specimen is **above 40 or is not determined**.

If the IC Ct value for such specimen differs from the $(IC\ Ct)_{av}$ value by more than 2, the result is regarded **equivocal**. A repeated analysis of the specimen, starting from the DNA extraction step is required.

10.6 The specimen is considered **positive**, i.e. containing **HPV18 DNA**, when Ct through **ROX** channel for this specimen is **less than or equals to 40.**

The specimen is considered **positive**, i.e. containing **HPV16 DNA**, when Ct through **HEX** channel for this specimen is **less than or equals to 40.**

10.7 If Ct value for **NC** through **ROX** or **HEX** channel **is less than or equal to 40,** it indicates the presence of contamination. In case of contamination, all positive results of this individual PCR test 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.

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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.