Instructions for Use

RealLine HPV HCR Screen
Str-Format

A QUALITATIVE ASSAY KIT FOR THE DETECTION OF DNA OF THE HIGH CARCINOGENIC RISK TYPES 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 AND 68 OF THE HUMAN PAPILLOMA VIRUS BY REAL TIME PCR

***In-vitro* Diagnostics**

CE

RealLine HPV HCR Screen (Str-Format)	**VBD8444**	**96 Tests**
valid from	**October 2019**	

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

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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:

Human Papilloma Viruses HPV are DNA-Viruses and in between are 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 %.

The **RealLine HPV HCR Screen** assay kit is designed for the detection of DNA of

- HPV type 16
- HPV type 18
- HPV type 31
- HPV type 33
- HPV type 35
- HPV type 39
- HPV type 45
- HPV type 51
- HPV type 52
- HPV type 56
- HPV type 58
- HPV type 59
- HPV type 66
- HPV type 68

The detection is subsequent to the isolation from clinical specimens using the extraction kits:
RealLine DNA-Express (REF VBC8899)
RealLine DNA-Extraction 3 (REF VBC8889)
RealLine Extraction 100 (REF VBC8896)

RealLine HPV HCR Screen kit can be used in clinical practice.

RealLine HPV HCR Screen kit is designed for the analysis of clinical materials: scrapings of the epithelial cells, semen, prostatic secretions, and urine. The assay is based on the real-time polymerase chain reaction (PCR) method with fluorescent detection of the amplified product.

The kit can be used in clinical practice.

The Str-Format Kit contains 2 x 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). The kit is compatible with real-time PCR systems such as CFX™96 (Bio-Rad, USA) and 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

Positive Control Sample (PC)	2 vials, 1 ml each
Ready Master Mix No.1 (RMM 1), lyophilized	96 test-tubes
Ready Master Mix No.2 (RMM 2), lyophilized	96 test-tubes
The kit is additionally supplied with optical-quality PCR-film	

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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 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. Increased fluorescence signal is due to the use of a specific for given CMV DNA sequence DNA hybridization probe that in the course of reaction binds with one of the DNA strands, also providing additional specificity of the method. DNA probe comprises of a fluorescent dye at the 5’ end and of fluorescence quencher at the 3’ end which significantly reduces the fluorescence intensity. During the polymerase synthesis of the complementary strand, due to the 5’-3’ nuclease activity of Taq DNA polymerase the probe is cleaved from the 5’-terminus and separation of the quencher and the dye occurs, resulting in the increase the fluorescence signal due to accumulation of the reaction product. Fluorescence intensity detected depends on initial quantity of pathogen DNA template in the sample.

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.

Two tubes (RMM 1 and RMM 2) are used in the analysis of the content of HPV HCR DNA for each sample.

4. SPECIFICATIONS

4.1. Sensitivity:

Sensitivity of detection of DNA HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 (on four samples, containing 100 DNA copies, prepared from Standard Reference Samples) equals 100 %.

4.2. Specificity:

Specificity of detection of DNA HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 (using Standard Reference Panel of negative DNA-extracts) equals 100 %.

4.3. Diagnostic evaluation:

Diagnostic sensitivity

Diagnostic sensitivity of DNA detection of human papilloma virus of high cancer risk: clinical trials, conducted on 95 positive samples showed a 100 % sensitivity (range 97% -100%, with 90% confidence level).

Diagnostic specificity

Diagnostic specificity of DNA detection of human papilloma virus of high cancer risk: clinical trials, conducted on 54 negative samples showed a 100 % specificity (range 95 % -100 %, with 90 % confidence level).

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 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 2, 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;
- 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

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 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

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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 Mixes (RMM 1 and RMM 2)** closed in the package at (18 – 25) °C for at least 30 minutes. Then open the package and cut the necessary number of tubes with RMM 1 and the same number of RMM 2 (including prepared samples and controls: 1 NC and 1 PC) with the knife. 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. Place and label the necessary tubes with RMM 1 and RMM 2 (according the number of prepared samples and controls.

Attention! *Labels should be placed on the lateral side of the tubes.*

9.1. Label the tubes with RMM for each specimen and control.

Attention! *Labels should be placed on the lateral side of the tubes.*

9.2. 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.

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

9.4. 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

9.5. Select the amplification detection channels.

For RMM 1:

- **FAM** – registration of IC DNA signal;
- **HEX** – registration of DNA signal for high carcinogenic types of HPV
- **ROX** – registration of DNA signal for high carcinogenic types of HPV

For RMM 2:

- **FAM** – registration of IC DNA signal
- **ROX** – registration of DNA signal for high carcinogenic types of HPV

9.6. 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** in each of RMM 1 and RMM 2 the program should detect:

- increase of the IC DNA amplification signal (channel **FAM**) and determine the threshold cycle, IC **Ct**;
- increase of the HPV DNA amplification signal (channels **HEX** and **ROX** in **RMM 1** and **ROX** in **RMM 2**) and determine the PC **Ct** value for each channel.

10.2 For **NC** in each of RMM 1 and RMM 2 the program should detect the increase of the amplification signal of IC DNA (channel **FAM**) and determine the threshold cycle, IC **Ct**. No **HEX** and **ROX** fluorescent increase should appear (*no HPV DNA amplification*) up to **Ct 40**.
When **Ct** value for NC through **ROX** or **HEX** channels for RMM 1 or **ROX** for RMM 2 **is less than or equal to 40,** this indicates the presence of contamination (see paragraph 9.7).

10.3 Analysis of the HPV HCR detection:

10.3.1. For each sample in RMM 1 and RMM 2 an increase of the amplification signal of **IC DNA** (channel **FAM**) should be detected and the IC **Ct** determined. .

10.3.2. Calculate (IC **Ct**)$_{av}$ as an average IC **Ct** of all analyzed samples (including PC and NC) independently for each of RMM 1 and RMM 2. 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.

10.4 The sample is considered **negative** (not containing HPV HCR DNA), if **Ct** value via **HEX** or **ROX** channels in RMM 1 and via **ROX** channel in RMM 2 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.

10.5 **RMM 1:**

The sample is considered **positive** for HPV, when **Ct** value via **HEX** and/or **ROX** channel for this sample is **less than or equals to 40**.

RMM 2:

Samples are considered **positive** for HPV, when **Ct** value via **ROX** channel for this sample is **less than or equals to 40**.

10.6 In case of contamination 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 samples of this run that were identified as positive. Samples that showed negative results in this run should be considered as negative.

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The sample is positive when a positive signal is detected in one or in both Mastermixes!
The occurrence of two or more different positive Genotypes in patient samples is often!

For genotyping of the HPV types use the RealLine HPV HCR Genotype kits (REF VBD8478, VBD8479, VBD8482) or ask us for more information**: techsupport@bioron.de**

11. STORAGE AND TRANSPORTATION

- Store and transport 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 for 50 µl aliquots 3 month at (-18 … -60) °C*
  Ready Master Mix (RMM): 3 months at (2 – 8) °C.

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.

Technical Support: techsupport@bioron.de