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**HPV 16&18 Real Time PCR Kit**

Cat. No.: TD-0030-02

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For use with ABI Prism<sup>®</sup>7000/7300/7500/7900; Smart CyclerII;  
iCycler iQ<sup>™</sup>4/iQ<sup>™</sup>5; Rotor Gene<sup>™</sup>2000/3000; Mx3000P/3005P;  
MJ-Option2/Chromo4 real time PCR systems

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For In Vitro Diagnostic Use Only

User Manual

## 1. Intended Use

HPV 16&18 real time PCR kit is used for the detection of HPV 16&18 in genital swabs samples by using real time PCR systems.

## 2. Introduction

The human papilloma virus (HPV) is one of the most common virus groups in the world to affect the skin and mucosal areas of the body. Different types of the HPV's are known to infect different parts of the body. Genital warts are known technically as condylomata acuminatum and are generally associated with two HPV types, numbers 6 and 11 and can be sexually transmitted. Two types of genital tract HPV in particular, HPV 16 and HPV 18, are known to cause up to 95% of cervical cancers, and new studies show that they may be linked to oral cancer as well. Both of these are genital viruses which are spread through sexual contact.

## 3. Principle of Real-Time PCR

The principle of the real-time detection is based on the fluorogenic 5' nuclease assay. During the PCR reaction, the DNA polymerase cleaves the probe at the 5' end and separates the reporter dye from the quencher dye only when the probe hybridizes to the target DNA. This cleavage results in the fluorescent signal generated by the cleaved reporter dye, which is monitored real-time by the PCR detection system. The PCR cycle at which an increase in the fluorescence signal is detected initially (Ct) is proportional to the amount of the specific PCR product. Monitoring the fluorescence intensities during Real Time allows the detection of the accumulating product without having to re-open the reaction tube after the amplification.

## 4. Product Description

HPV 16&18 real time PCR kit contains a specific ready-to-use system for the detection of HPV 16&18 by polymerase chain reaction in the real-time PCR system. The master contains reagents and enzymes for the specific amplification of HPV 16&18 DNA. Fluorescence is emitted and measured by the real time systems' optical unit during PCR. The detection of amplified HPV 16&18 DNA fragment is performed in fluorimeter **channel FAM** with the fluorescent quencher BHQ1. DNA extraction buffer is available in the kit and genital swabs samples are used for DNA extraction. In addition, the kit contains a system to identify possible PCR inhibition by measuring the VIC/JOE fluorescence of the internal control (IC). An external positive control ( $1 \times 10^7$  copies/ml) contained, allows the determination of the gene load. For further information, please refer to section 10.3 Quantitation.

## 5. Kit Contents

Ref.	Type of Reagent	Presentation	25rxns
1	DNA Extraction Buffer	1 vial, 1.8ml	
2	HPV Serotype 16 Reaction Mix	1 vial, 950µl	
3	HPV Serotype 18 Reaction Mix	1 vial, 950µl	
4	PCR Enzyme Mix	1 vial, 22µl	
5	Molecular Grade Water	1 vial, 400µl	
6	Internal Control (IC)	1 vial, 55µl	
7	HPV16&18 Positive Control( $1 \times 10^7$ copies/ml)	1 vial, 60µl	

## 6. Storage

- All reagents should be stored at -20°C. Storage at +4°C is not recommended.
- All reagents can be used until the expiration date indicated on the kit label.
- Repeated thawing and freezing (>3x) should be avoided, as it may reduce the assay sensitivity.

- Cool all reagents during the working steps.
- Reaction Mix should be stored in the dark.

## **7. Additionally Required Materials and Devices**

- Biological cabinet
- *Real time* PCR system
- Desktop microcentrifuge for “eppendorf” type tubes (RCF max. 16,000 x g)
- Vortex mixer
- *Real time* PCR reaction tubes/plates
- Cryo-container
- Pipets (0.5 µl – 1000 µl)
- Sterile filter tips for micro pipets
- Sterile microtubes
- Disposable gloves, powderless
- Biohazard waste container
- Refrigerator and freezer
- Tube racks

## **8. Warnings and Precaution**

Carefully read this instruction before starting the procedure.

- For in vitro diagnostic use only.
- This assay needs to be carried out by skilled personnel.
- Clinical samples should be regarded as potentially infectious materials and should be prepared in a laminar flow hood.
- This assay needs to be run according to Good Laboratory Practice.
- Do not use the kit after its expiration date.
- Avoid repeated thawing and freezing of the reagents, this may reduce the sensitivity of the test.
- Once the reagents have been thawed, vortex and centrifuge briefly the tubes before use.
- Prepare quickly the Reaction mix on ice or in the cooling block.
- Set up two separate working areas: 1) Isolation of the RNA/ DNA and 2) Amplification/ detection of amplification products.
- Pipets, vials and other working materials should not circulate among working units.
- Use always sterile pipette tips with filters.
- Wear separate coats and gloves in each area.
- Do not pipette by mouth. Do not eat, drink, smoke in laboratory.
- Avoid aerosols

## **9. Sample Collection, Storage and Transport**

- Collect samples in sterile tubes;
- Specimens can be extracted immediately or frozen at -20°C to -80°C.
- Transportation of clinical specimens must comply with local regulations for the transport of etiologic agents

## **10. Procedure**

### **10.1 DNA-Extraction**

DNA extraction buffer is supplied in the kit, please thaw the buffer thoroughly and spin down briefly in the centrifuge before use.

- 1) Wash the genital swabs in 1.0ml normal saline and vortex vigorously. Centrifuge at 13000rpm for 5

- minutes. Carefully remove and discard supernatant from the tube without disturbing the pellet.
- 2) Add 1.0ml normal saline and suspend the pellet with vortex vigorously. Centrifuge at 13000rpm for 5 minutes. Carefully remove and discard supernatant from the tube without disturbing the pellet.
  - 3) Add 50µl DNA extraction buffer, close the tube then resuspend the pellet with vortex vigorously. Spin down briefly in a table centrifuge.
  - 4) Incubate the tube for 10 minutes at 100°C.
  - 5) Centrifuge the tube at 13000rpm for 5 minutes. The supernatant contains the DNA extracted and can be used for PCR template.

**Attention:**

- A. During the incubation, make sure the tube is not open. Since the vapor will volatilize into the air and may cause contamination if the sample is positive.
- B. The extraction sample should be used in 3 hours or stored at -20°C for one month.
- C. DNA extraction kits are available from various manufacturers. You can also use your own extraction systems or the commercial kit depending on the yield. For DNA extraction, please comply with the manufacturer's instructions.

**10.2 Internal Control**

It is necessary to add internal control (IC) in the reaction mix. Internal Control (IC) allows the user to determine and control the possibility of PCR inhibition.

Add the internal control (IC) 1µl/rxn and the result will be shown in the VIC/JOE channel.

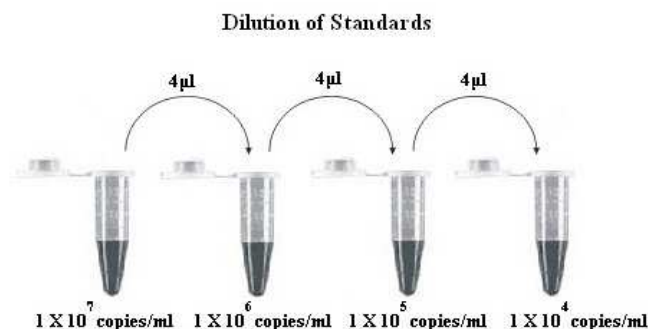
**10.3 Quantitation**

The kit can be used for **quantitative** or **qualitative** real-time PCR. A positive control defined as  $1 \times 10^7$  copies/ml is supplied in the kit.

**For performance of quantitative real-time PCR, Standard dilutions must prepare first as follow. Molecular Grade Water is used for the diluent.**

**Dilution is not need for performance of qualitative real-time PCR.**

Take positive control ( $1 \times 10^7$  copies/ml) as the starting high standard in the first tube. Respectively pipette **36ul** Molecular Grade Water into next three tubes. Do three dilutions as the following figures:



To generate a standard curve on the real-time system, all four dilution standards should be used and defined as standard with specification of the corresponding concentrations.

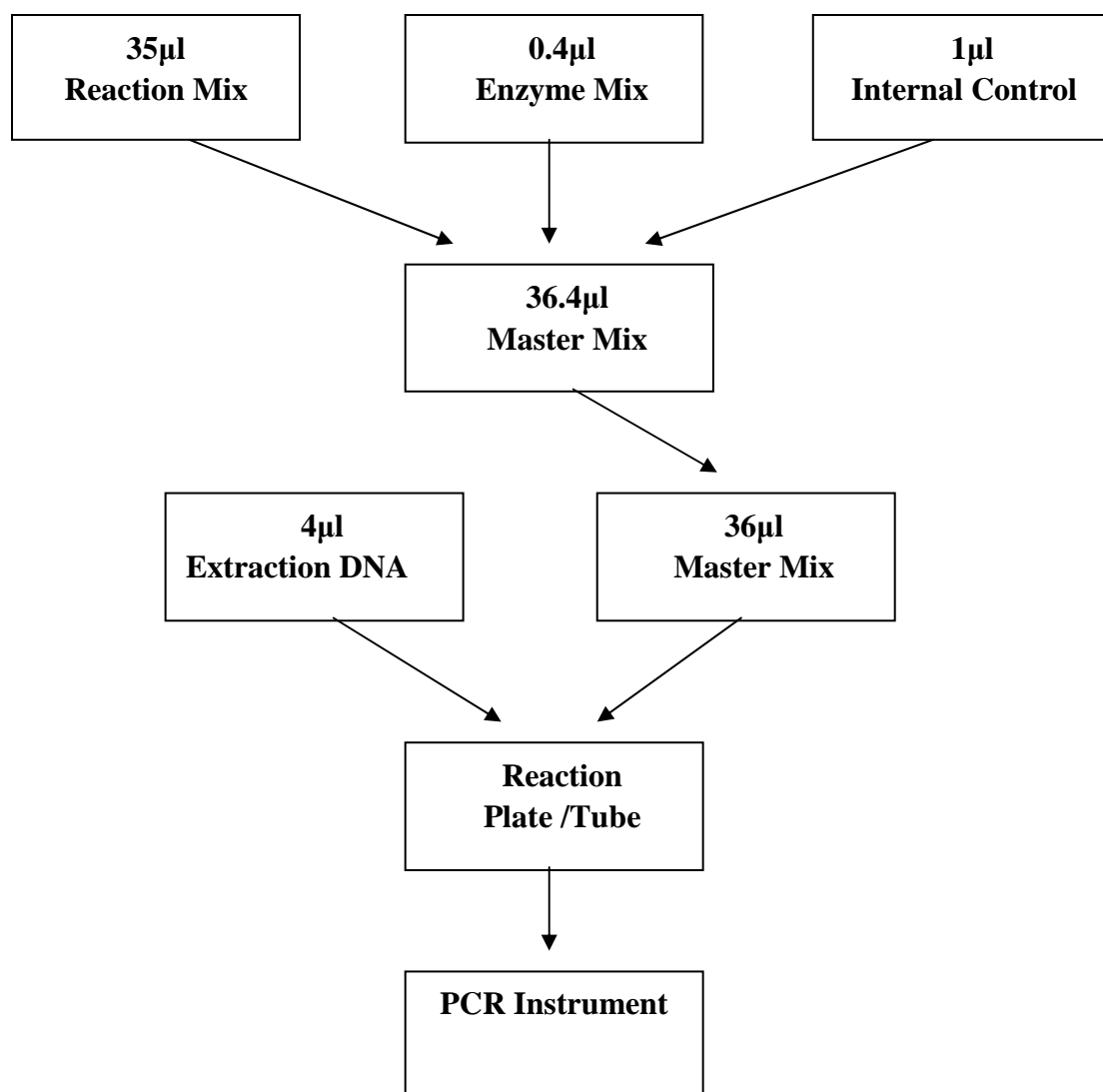
**Attention:**

- A. Mix thoroughly before next transfer.
- B. The positive control ( $1 \times 10^7$  copies/ml) contains high concentration of the target DNA. Therefore, be

Careful during the dilution in order to avoid contamination.

#### 10.4 PCR Protocol

The Master Mix volume for each reaction should be pipetted as follow:



- 1) The volumes of Reaction Mix and Enzyme Mix per reaction multiply with the number of samples, which includes the number of controls, standards, and sample prepared. Molecular Grade Water is used as the negative control. For reasons of unprecise pipetting, always add an extra virtual sample. (**n: the number of reaction** ).

Reaction Volume	Master Mix Volume
35µl Reaction Mix	$35\mu\text{l} \times (n+1)$
0.4µl Enzyme Mix	$0.4\mu\text{l} \times (n+1)$
1µl Internal control (IC)	$1\mu\text{l} \times (n+1)$

Mix completely then spin down briefly in a centrifuge.

- 2) Pipet **36µl HPV Serotype 16** Master Mix with micropipets of sterile filter tips to each *Real time* PCR reaction plate/tubes. Separately add **4µl** DNA sample template, positive and negative controls to different reaction plate/tubes. Immediately close the plate/tubes to avoid contamination.
- 3) Pipet **36µl HPV Serotype 18** Master Mix with micropipets of sterile filter tips to each *Real time* PCR reaction plate/tubes. Separately add **4µl** DNA sample template, positive and negative controls to

different reaction plate/tubes. Immediately close the plate/tubes to avoid contamination.

- 4) Spin down briefly in order to collect the Master Mix in the bottom of the reaction tubes.
- 5) Perform the following protocol in the instrument:

**37°C for 2 min, 1 cycle;**



**94°C for 2 min, 1 cycle;**



**93°C for 15 sec, 60°C for 60 sec, 40 cycles.**

**Fluorescence is measured at 60°C**

- 5) If you use ABI Prism<sup>®</sup> system, please choose “**none**” as **passive reference** and **quencher**.

### **11. Data Analysis and Interpretation**

The following results are possible:

- 1) A signal is detected in channel FAM of the **HPV Serotype 16** Master Mix. **The result is positive: The sample contains HPV Serotype 16 DNA.**

In this case, the detection of a signal in channel VIC/JOE (Internal control) is dispensable, as high initial concentrations of HPV Serotype16 DNA can lead to a reduced or absent fluorescence signal of the internal control (competition).

- 2) A signal is detected in channel FAM of the **HPV Serotype 18** Master Mix. **The result is positive: The sample contains HPV Serotype 18 DNA.**

In this case, the detection of a signal in channel VIC/JOE (Internal control) is dispensable, as high initial concentrations of HPV Serotype18 DNA can lead to a reduced or absent fluorescence signal of the internal control (competition).

- 3) In channel FAM no signal is detected, at the same time, a VIC/JOE signal from the Internal Control appears. **The sample does not contain any HPV 16&18 DNA. It can be considered negative.**

In the case of a negative HPV 16&18 PCR the detected signal of the internal control rules out the possibility of PCR inhibition.

- 4) Neither in channel FAM nor in channel VIC/JOE is a signal detected. **A diagnostic statement can not be made.** Inhibition of the PCR reaction.

Gentaur Molecular Products  
Voortstraat 49  
1910 Kampenhout, Belgium