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# Uterine Cervix Cancer of High-risk HPV Genotype Related Real Time PCR Kit
## User Manual
## For In Vitro Diagnostic Use Only

REF TD-0031-02

*For use with ABI Prism®7000/7300/7500/7900/Step One Plus; iCycler iQ™4/iQ™5; Smart Cycler II;Bio-Rad CFX 96;Rotor Gene™6000; Mx3000P/3005P;MJ-Option2/Chromo4; LightCycler®480 Instrument*


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**1. Intended Use**

Uterine Cervix Cancer of High-risk HPV Genotype Related Real-time PCR kit is used for the detection of 13 Types of High-risk HPV Genotypes in genital swabs samples by using real time PCR systems.

**2. 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 is proportional to the amount of the specific PCR product. Monitoring the fluorescence intensities in real-time allows the detection of the accumulating product without having to re-open the reaction tube after the amplification.

**3. Product Description**

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. 13 types of genital tract HPV in particular, HPV 16 ,18, 31,33,35,39,45,51,52,56,58,59, 68 are known to cause up to 99% of cervical cancers, and new studies show that they may be linked to oral cancer as well. All of these are genital viruses which are spread through sexual contact. Uterine Cervix Cancer of High-risk HPV Genotype Related Real-time PCR kit contains a specific ready-to-use system for the detection of 13 Types of High-risk HPV Genotypes in genital swabs samples by polymerase chain reaction in the real-time PCR system. The master contains reagents and enzymes for the specific amplification of HPV DNA. Fluorescence is emitted and measured by the real time systems´ optical unit during PCR. The detection of amplified HPV DNA fragment is performed in fluorimeter **channel FAM and HEX/VIC/JOE** with the fluorescent quencher BHQ1. DNA extraction buffer is available in the kit and genital swabs samples are used for DNA extraction. An external positive control contained all 13 types of high risk HPV partial sequence.

**4. Kit Contents**

| Ref. | Type of Reagent | Presentation 25rxns |
|---|---|---|
| 1 | DNA Extraction Buffer | 1 vial, 1.8ml |
| 2 | HPV 16,56 Reaction Mix | 1 vial, 950µl |
| 3 | HPV 18,45 Reaction Mix | 1 vial, 950µl |
| 4 | HPV 35,59 Reaction Mix | 1 vial, 950µl |
| 5 | HPV 39,51 Reaction Mix | 1 vial, 950µl |
| 6 | HPV 58,52 Reaction Mix | 1 vial, 950µl |
| 7 | HPV 31 ,IC Reaction Mix | 1 vial, 950µl |
| 8 | HPV 33 Reaction Mix | 1 vial, 950µl |
| 9 | HPV 68 Reaction Mix | 1 vial, 950µl |
| 10 | PCR Enzyme Mix | 1 vial, 88µl |
| 11 | Molecular Grade Water | 1 vial, 400µl |
| 12 | HPV Positive Control | 1 vial, 100µl |

**Analysis sensitivity: $5\times10^3$ copies/ml**

**Note:** Analysis sensitivity depends on the sample volume, elution volume, nucleic acid extraction methods and other factors .If you use the DNA extraction buffer in the kit, the analysis sensitivity is the same as it declares. However, when the sample volume is dozens or even hundreds of times greater than elution volume by some concentrating method, it can be much higher.

**5. 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 this may reduce the sensitivity of the assay.
- Cool all reagents during the working steps.
- Super Mix should be stored in the dark.

**6. Additionally Required Materials and Devices**

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

**7. ⚠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.

**8. 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.

**9. Procedure**

**9.1 DNA-Extraction**

DNA extraction buffer is supplied in the kit, please thaw the buffer thoroughly and spin down briefly in the centrifuge before use. You may use your own extraction systems or commercial kit**s.**

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 vortex for 10 seconds. 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.

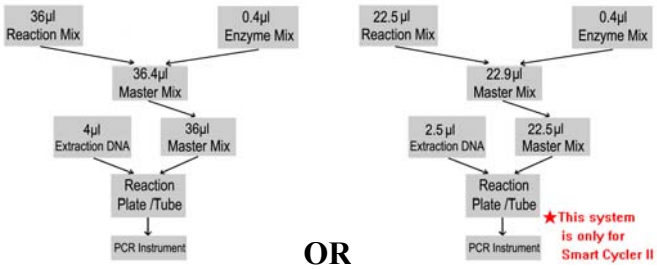
**9.2 Internal control**

MNBH gene is detected as an internal control. All clinical samples should exhibit MNBH positive, thus indicating the presence of sufficient nucleic acid from human MNBH gene. Failure to detect MNBH in any of clinical samples may indicate that:

1) Improper extraction of nucleic acid.
2) Absence of sufficient human cellular material in sample.
3) Improper assay set up and execution
4) Reagent or equipment malfunction.

**9.3 PCR Protocol**

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




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. Mix completely then spin down briefly in a centrifuge.
2) Pipet **36µl (22.5µl for SmartCycler II)** Master Mix with micropipets of sterile filter tips to each real time PCR reaction plate/tubes. Separately add **4µl (2.5µl for SmartCycler II)** DNA sample template, positive and negative controls to different reaction plate/tubes. Immediately close the plate/tubes to avoid contamination.
3) Spin down briefly in order to collect the Master Mix in the bottom of the reaction tubes.
4) Perform the following protocol in the instrument:

| | |
|---|---|
| 94°C for 2min | 1cycle |
| 93°C for 10sec, 62°C for 31sec ( Fluorescence measured at 60°C) | 40cycles |

| Selection of fluorescence channels | |
|---|---|
| FAM | Target Nucleic Acid |
| HEX/VIC/JOE | Target Nucleic Acid |

5) ⚠ If you use ABI Prism® system, please choose **"none"** as **passive reference** and **quencher.**

**10. Threshold setting:** just above the maximum level of molecular grade water.

**11. Quality control:** Negative control and positive control must be performed correctly, otherwise the sample results is invalid.

| | Channel | Molecular Grade Water (Negative control) | | Positive Control | |
|---|---|---|---|---|---|
| | Master Mix | FAM | HEX | FAM | HEX |
| 1 | HPV 16,56 Reaction Mix | UNDET | UNDET | Ct≤35 | Ct≤35 |
| 2 | HPV 18,45 Reaction Mix | UNDET | UNDET | Ct≤35 | Ct≤35 |
| 3 | HPV 35,59 Reaction Mix | UNDET | UNDET | Ct≤35 | Ct≤35 |
| 4 | HPV 39,51 Reaction Mix | UNDET | UNDET | Ct≤35 | Ct≤35 |
| 1 | HPV 58,52 Reaction Mix | UNDET | UNDET | Ct≤35 | Ct≤35 |
| 2 | HPV 31 ,IC Reaction Mix | UNDET | UNDET | Ct≤35 | —— |
| 3 | HPV 33 Reaction Mix | UNDET | UNDET | Ct≤35 | —— |
| 4 | HPV 68 Reaction Mix | UNDET | UNDET | Ct≤35 | —— |

**12. Data Analysis and Interpretation**

1）The Ct value shows ≤38. **The result is positive: The sample contains some serotype of HPV DNA.** The following results are possible:

| Reaction Mix | Channel | Ct value | Result Analysis |
|---|---|---|---|
| HPV 16,56 | FAM | ≤38 | The sample contains HPV Serotype 16 DNA |
| | HEX/VIC/JOE | ≤38 | The sample contains HPV Serotype 56 DNA |
| HPV 18,45 | FAM | ≤38 | The sample contains HPV Serotype 18 DNA |
| | HEX/VIC/JOE | ≤38 | The sample contains HPV Serotype 45 DNA |
| HPV 35,59 | FAM | ≤38 | The sample contains HPV Serotype 35 DNA |
| | HEX/VIC/JOE | ≤38 | The sample contains HPV Serotype 59 DNA |
| HPV 39,51 | FAM | ≤38 | The sample contains HPV Serotype 39 DNA |
| | HEX/VIC/JOE | ≤38 | The sample contains HPV Serotype 51 DNA |
| HPV 58,52 | FAM | ≤38 | The sample contains HPV Serotype 58 DNA |
| | HEX/VIC/JOE | ≤38 | The sample contains HPV Serotype 52 DNA |
| HPV 31,IC | FAM | ≤38 | The sample contains HPV Serotype 31 DNA |
| | HEX/VIC/JOE | —— | — — |
| HPV 33 | FAM | ≤38 | The sample contains HPV Serotype 33 DNA |
| | HEX/VIC/JOE | —— | — — |
| HPV 68 | FAM | ≤38 | The sample contains HPV Serotype 68 DNA |
| | HEX/VIC/JOE | —— | — — |

2) The Ct value shows 38~40, please repeat again. If the result still shows 38~40, it can be considered negative. But the clinical samples in channel of HEX/VIC/JOE in **HPV 31,IC Reaction Mix** should be positive. Otherwise, the negative result of the sample is invalid. Please refer to section 9.2 in detail.

3) In channel FAM or HEX/VIC/JOE no signal is detected in any one of 13 Serotypes HPV Master Mix. The sample does not contain any one of 13 Serotypes HPV. It can be considered negative.

For further questions or problems，please contact our technical support at **trade@liferiver.com.cn**