1. Intended Use
High-Risk Human Papillomaviruses (HPV) and Genotyping 16 & 18 Real-Time PCR Kit is used for the detection of 14 high risk HPV viruses to support the diagnosis of HPV-caused cervical cancer. Using fluorescent PCR detection method, this kit can detect specific DNA fragments of HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 in secretion of unregulated tracts and in cervical cells. Among them, HPV subtypes 16 and 18 are detected separately.

2. Principle of Real-Time PCR
The principle of real-time PCR detection is based on the fluorogenic 5’ nucleotide assay. During 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 and the target DNA are hybridized. This cleavage results in the fluorophore generated by the cleaved reporter dye, which is monitored real time by the PCR detection system. PCR cycle at which an increase in fluorescence signal is detected is initially proportional to the amount of the specific PCR product. Monitoring the fluorescence intensities in real time allows the detection of the accumulating product with reaction tube not being reopened after amplification.

3. Product Description
Different HPV subtypes are known to infect different parts of the body. High-Risk Human Papillomavirus (HPV) and Genotyping 16 & 18 Real-Time PCR Kit contains a specific ready-to-use system for the detection of HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 subtypes with real time PCR system and Taqman technique. It can detect the specific DNA fragments in HPV. The Master Mix contains reagents and enzymes for the specific amplification of HPV DNA. Fluorescence is emitted and measured by real time PCR system’s optical unit during PCR. The detection of amplified HPV DNA fragment is performed in fluorometer chambers, MNBH® system, please choose the appropriate fluorophore and quencher. The Master Mix contains reagents and enzymes for the specific amplification of HPV DNA. Fluorescence is emitted and measured by real time PCR system’s optical unit during PCR. The detection of amplified HPV DNA fragment is performed in fluorometer chambers.

4. Kit Contents

<table>
<thead>
<tr>
<th>Type of Reagent</th>
<th>Presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 DNA Extraction Buffer</td>
<td>2 vials, 1.4ml</td>
</tr>
<tr>
<td>2 HPV Reaction Mix</td>
<td>1 vial, 950μl</td>
</tr>
<tr>
<td>3 PCR Enzyme Mix</td>
<td>1 vial, 12μl</td>
</tr>
<tr>
<td>4 Molecular Grade Water</td>
<td>1 vial, 400μl</td>
</tr>
<tr>
<td>5 HPV Positive Control</td>
<td>1 vial, 400μl</td>
</tr>
</tbody>
</table>

5. Storage
- All reagents should be stored at -20°C. Storage at 4°C is not recommended.
- All reagents can be used till expiration date indicated on the kit label.
- Repeated thawing and freezing (>3x) should be avoided as this may reduce assay sensitivity.
- Keep all reagents cool during working steps.
- Reaction Mix should be stored in the dark.

6. Additional 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/pipettes
- Cryo-container
- Pipettes (0.5 μl - 1000 μl)
- Sterile filter tips for micro pipettes
- Sterile microtubes
- Disposable powderless gloves
- Biohazard waste container
- Refrigerator and freezer
- Tube racks

7. Warning and Precaution
- Carefully read this instruction before starting the procedure.
- This assay needs to be carried out by skilled personnel.
- Clinical samples should be regarded as potentially infectious and thus be prepared in a laminar flow hood.
- This assay needs to be run according to Good Laboratory Practice.
- Do not use the kit after expiration date.
- Avoid repeated thawing and freezing of the reagents as this may reduce the sensitivity of the test.
- Once the reagents have been thawed, vortex and briefly centrifuge the tubes before use.
- Prepare quickly the Reaction Mix on ice or in the cooling block.
- Do not use two separate working areas: 1) isolation of the RNA: DNA and 2) Amplification/ detection of amplification products.
- Do not calculate pipettes, vials and other working materials among working units.
- Use always sterile pipette tips with filter.
- Wear separate coats and gloves in each area.
- Do not use pipette by mouth. Do not eat, drink or 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 included in the kit. Please thaw the buffer thoroughly and spin down briefly in the centrifuge before use. You may use your own extraction system or commercial kit.

9.1.1 Genital swab and cervical cell samples
- 1) A cervical sample is used to gently scrape cells from the cervix. The sample should be put into a closed sterile glass tube.
- 2) Wash the sample in 1ml normal saline and vortex vigorously. Centrifuge at 13,000rpm for 5 minutes. Carefully remove and discard supernatant from the tube without disturbing the pellet.
- 3) Add 1ml normal saline and suspend the pellet with vigorous vortex. Centrifuge at 13,000rpm for 5 minutes. Carefully remove and discard supernatant from the tube without disturbing the pellet.
- 4) Add 50μl DNA extraction buffer, close the tube and then vortex for 10 seconds. Spin down briefly in a table centrifuge.
- 5) Incubate the tube for 10 minutes at 100°C.
- 6) Centrifuge the tube at 13,000rpm for 5 minutes. The supernatant contains the DNA extracted and can be used for PCR template.

Attention: During the incubation, make sure the tube is not open. Since the vapor will go in and may cause contamination if the sample is positive.

B) The extraction sample should be used within 3 hours or stored at -20°C for one month.

9.2 Internal control
MNBH gene is detected as an internal control. All clinical samples should exhibit MNBH positive, indicating the presence of sufficient nucleic acid from human MNBH gene. Failure to detect MNBH in any of clinical samples may indicate:
- Improper extraction of nucleic acid.
- Absence of sufficient human cellular material in sample.
- Improper assay set up or execution.
- Reagent or equipment malfunction.

9.3 PCR protocol
- The Master Mix volume for each reaction should be pipetted as follows:
  1) Multiply the volumes of Reaction Mix and Enzyme Mix per reaction with the number of samples, which includes the number of controls and samples prepared. Molecular Grade Water is used as the negative control. For reasons of imprecise pipetting, always add an extra virtual sample. Mix completely and then spin down briefly in a centrifuge.
  2) Pipette 3μl Master Mix with micropipettes of sterile filter tips to each real time PCR reaction well. Separately add 4μl DNA sample template, positive and negative controls to different reaction well.

Immediate close the well to avoid contamination.

3) Spin down briefly in order to collect the Master Mix in the bottom of the reaction wells.

4) Run the following protocol in the instrument:

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Steps</th>
</tr>
</thead>
<tbody>
<tr>
<td>94°C for 2min</td>
<td>1 cycle</td>
</tr>
<tr>
<td>93°C for 10sec, 62°C (Lower 0.2°C for each cycle) for 31sec</td>
<td>15 cycles</td>
</tr>
<tr>
<td>93°C for 10sec, 61°C for 31sec</td>
<td>40 cycles</td>
</tr>
<tr>
<td>(Fluorescence measured at 61°C)</td>
<td></td>
</tr>
</tbody>
</table>

5) If you use ABI Prism system, please choose “none” as passive reference and quencher.

6) If you use LightCycler®480 system, please do color compensation before detection.

10. Baseline Setting: Just above the maximum level of molecular grade water.

11. Quality Control

<table>
<thead>
<tr>
<th>Controls</th>
<th>FAM</th>
<th>HEX</th>
<th>CY5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular Grade Water (Negative Control)</td>
<td>UNDET</td>
<td>UNDET</td>
<td>UNDET</td>
</tr>
<tr>
<td>Positive Control</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Ct ≤ 35

12. Data Analysis and Interpretation
1) Positive result: The sample contains some serotype of HPV DNA. The following results are possible:

<table>
<thead>
<tr>
<th>Genotype</th>
<th>FAM</th>
<th>HEX</th>
<th>CY5</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1 – C38</td>
<td>UNDET</td>
<td>UNDET</td>
<td>At least one of HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68</td>
</tr>
<tr>
<td>C15 – C22</td>
<td>UNDET</td>
<td>UNDET</td>
<td>HPV 16</td>
</tr>
<tr>
<td>C31 – C33</td>
<td>UNDET</td>
<td>UNDET</td>
<td>C5 ≤ 22</td>
</tr>
<tr>
<td>C5 ≤ 22</td>
<td>UNDET</td>
<td>UNDET</td>
<td>Lower than detection limit or negative</td>
</tr>
</tbody>
</table>

For further questions, please contact our technical support at info@liferiverbiotech.com.