USER MANUAL
Real-time PCR assay for detection, typing and quantification of human papillomavirus (HPV)

The HPV-QUANT-15® quantitative PCR Kit

General information

Intended use:
The HPV-QUANT-15® quantitative PCR Kit is in vitro DNA test, which is intended for the specific identification and quantification of low- and high-risk (in regard to oncogenic properties) human papillomaviruses including:
- low-risk HPV types 6 and 11 without differentiation
- high-risk HPV types 16, 31, 33, 35, 52, 58 without differentiation
- HPV types 18, 39, 45, 59 without differentiation
- HPV type 56
- HPV type 51
- HPV type 68

Method:
Real-time polymerase chain reaction; qualitative and quantitative multiplex detection. The quantification based on two approaches: absolute and relative. The absolute quantification approach take into account the threshold cycle (Cp) value which then used for viral DNA copy number evaluation. The relative quantification approach use normalization algorithm which allow to compare the viral DNA copy number and number of human genomes (sample intake control) which correlate to the number of cells in a sample. The least approach consider the sample-to-sample variability.

Attention! Considering the proper sample intake, the clinically relevant viral load is not less than $10^3$ HPV DNA copies per sample. This value characterized as high level infection, which can cause the cervical cancer. For this reason the relative values are restricted by the software. The result considered to be negative if it falls out of the clinically relevant range.

Samples:
Swab from urethra, cervix, or posterolateral vaginal wall.

DNA extraction:
We recommend using DNA-Technology PREP-NA-PLUS and PREP-GS-PLUS Kits for DNA extraction.

Features:
Simultaneous detection of several DNA-targets in one tube (multiplex)

Controls:
PCR-Mix contains internal control (IC). IC is needed for extraction and PCR quality assessment. PCR-Mix contains sample intake control (SIC). SIC is needed for sample quality assessment. When estimating the relative number of HPV SIC value is used for normalization. PCR-Mix contains Marker. Marker is a free dye used to control the proper orientation of strips.

Devices:
DNA-Technology thermal cyclers with detection unit (real-time thermal cyclers): DTlite\(^1\), DTprime\(^2\), DT-96; software version 7.3.5.84 or higher.

Number of tests: 48

\(^1\) - 4S1, 4S2, 5S2, 6S1, 6S2 models
\(^2\) - 4M1, 4M3, 4M6, 5M1, 5M3, 5M6, 6M1, 6M3, 6M6 models
**Content**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paraffin sealed PCR-mix</td>
<td>20 µL, 24 8-tube-strips</td>
</tr>
<tr>
<td>MAX Taq-polymerase solution</td>
<td>960 µL, 2 tubes</td>
</tr>
<tr>
<td>Mineral oil</td>
<td>960 µL, 4 tubes</td>
</tr>
<tr>
<td>Positive control sample (“C+”) HPV quant-15</td>
<td>150 µL, 1 tube</td>
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</table>

**Strip content, colour codes and detection channels**

<table>
<thead>
<tr>
<th>Nº of the tube in a strip</th>
<th>Dye label/detection channel</th>
<th>Colour of the PCR-mix</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td>4,8</td>
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</tr>
</tbody>
</table>

**Procedure**

1. **Preparing the PCR**
   1.1 Mark the required number of 8-tube PCR-strips for each sample and control to be tested.
   
   **Note.** One strip contain PCR-mixes for two samples testing.
   
   **Example:** for testing of 2 samples, mark 1 strip for samples and 1 strip for “C-“ and “C+”. The resulting number of strips is 2.

<table>
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<td>Sample 2</td>
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1.2 Mix the MAX Taq-polymerase solution thoroughly (3-5 sec), then spin briefly (1-3 sec).
1.3 Add 10 µL of Taq-polymerase MAX solution into each tube. Avoid paraffin layer break.
1.4 Add one drop (~20 µl) of mineral oil into each tube of the strip. Close strips tightly.
1.5 Add 5,0 µL of DNA sample into corresponding strip. Avoid paraffin layer break. Open the tube, add DNA sample, then close the tube before proceeding to the next DNA sample to prevent contamination. Use filter tips.
1.6 Add 5,0 µL of “C-“ which passed whole DNA extraction procedure and “C+“ into corresponding strip. Avoid paraffin layer break.
1.7 Spin strips briefly (1-3 sec).
1.8 Set the strips to Real-time PCR instrument. Try to place strips in the center of thermoblock.
1.9 Launch RealTime_PCR software and choose the Device handling mode. Download «HPV QUANT.ini» file if you do this test for the first time. In subsequent runs add the “HPV quant-15” test to the protocol, specify the number and ID’s of the samples, specify the position of the strips in the thermal unit (p. 1.8) and run PCR.

2. **Registration and interpretation of the PCR results** held in automatic mode.

**Storage and handling requirements**

All components of The Kit must be stored at 2-8 °C during the storage period.
Expiry date – 6 months since the date of production.

Contact our customer service by quality issues of The HPV-QUANT-15® quantitative PCR Kit: 115587, Moscow, Varshavskoye sh. 125g building 6, DNA Technology, LLC.
Phone/Fax: +7(495)9804555
Technical support: +7 (800) 200-75-15, E-mail: hotline@dna-technology.ru, www.dna-technology.ru

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