HPV QUANT-15 Quantitative REAL-TIME PCR Kit

REF R1-P316-S3/4EU

The full instruction № 421.14.03.16 (HPV QUANT-15) is available on the "DNA-Technology" Company's web-page:
http://www.dna-technology.ru/eng/dnaproducts/reagents/

General information

Intended use:
The HPV QUANT-15 Quantitative REAL-TIME 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:
Swabs from the urethra, the surface of the cervix, or the cervical canal.

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 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:
The automatic analysis for HPV QUANT-15 Quantitative REAL-TIME PCR Kit is available on "DNA-Technology" made DTitl\(\text{\textdegree}\), DTprime\(\text{\textdegree}\) and DT-96 REAL-TIME Thermal Cyclers; software version is not lower than 7.3.5.84; the current version of the software is available for download at http://www.dna-technology.ru/eng/support/

Overall time needed to perform the analysis (excluding sample preparation procedure):
from 2.5 hours.

The number of tests:
48

1 - supported by 4S1, 4S2, 5S1, 5S2, 6S1, 6S2 instruments.
2 - supported by 4M1, 4M3, 4M6, 5M1, 5M3, 5M6, 6M1, 6M3, 6M6 instruments.
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>500 µL 4 tubes</td>
</tr>
<tr>
<td>• Mineral oil</td>
<td>1.0 mL 4 tubes</td>
</tr>
<tr>
<td>• Positive control sample HPV quant-15</td>
<td>150 µL 1 tube</td>
</tr>
</tbody>
</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>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fam</td>
<td>Hex</td>
</tr>
<tr>
<td>1,5</td>
<td>HPV</td>
<td>16</td>
</tr>
<tr>
<td>2,6</td>
<td>HPV</td>
<td>18</td>
</tr>
<tr>
<td>3,7</td>
<td>HPV</td>
<td>51</td>
</tr>
<tr>
<td>4,8</td>
<td>SIC</td>
<td></td>
</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>
<thead>
<tr>
<th>Sample 1</th>
<th>Tubes 1 – 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 2</td>
<td>Tubes 5 – 8</td>
</tr>
<tr>
<td>“C-”</td>
<td>Tubes 1 – 4</td>
</tr>
<tr>
<td>“C+”</td>
<td>Tubes 5 – 8</td>
</tr>
</tbody>
</table>

1.2 Mix the MAX Taq-polymerase solution thoroughly (3-5 sec), then spin briefly (1-3 sec).
1.3 Add 10 µL of MAX Taq-polymerase 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_en.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.

Shipping, storage and handling requirements

All kit components must be stored at the temperature between 2 °C and 8 °C and out of light during the storage period.
Transportation of kit’s components can be held by all types of roofed transport at the temperatures corresponding to the storage conditions of individual reagents, included in the kit.
Shelf-life – 6 months from the date of Quality Control Department approval in compliance with all transportation, storage and operation conditions.
Contact our customer service department regarding issues of quality HPV QUANT-15 Quantitative REAL-TIME PCR Kit:
Phone: +7 (495) 640-16-93,
Phone/Fax: +7 (495) 640-17-71.
E-mail: hotline@dna-technology.ru, www.dna-technology.ru
Address: 117587, Moscow, Varshavskoye sh., 125g building 6, DNA Technology.