# Human Papillomavirus [HPV] 14 high risk Viruses Real-time PCR Kit

**(For Qualitative Detection)**

IVD

-25°C -15°C

**REF 8024**



## INTENDED USE

HPV 14 high-risk virus Real-time PCR Kit is an in vitro nucleic acid amplification kit for the detection of HPV 14 high risk HPV virus specific DNA. Among 14 high risk HPV viruses, it detects and differentiates HPV 16 & 18 specific DNA.

## KIT CONTENTS

| Components | No. of vials | Volume Per vials |
|---|---|---|
| Probe PCR Master Mix | 1 | 250µl |
| HPV 16 & 18 Primer Probe Mix [HPV 16 & 18 PP mix] | 1 | 125µl |
| HPV 12 high risk viruses Primer Probe Mix [HPV 12 PP mix] | 1 | 65µl |
| Endogenous control Primer Probe Mix [Endogenous PP mix] | 1 | 65µl |
| Positive control mix [QS1] | 1 | 250µl |
| Water, PCR grade | 1 | 4ml |

## STORAGE

1. The kit is shipped on gel ice. Upon arrival, all components should be stored in -20°C. They are stable until the expiration date stated on the label.
2. Repeated thawing and freezing should be avoided, as this might affect the performance of the assay.
3. If the reagents are to be used only intermittently, they should be frozen in aliquots. Storage at 2 to 8°C should not exceed a period of 5 hours.

## PRODUCT DESCRIPTION

HPV 14 high-risk virus Real-time PCR Kit constitutes a ready-to-use system for the detection of HPV 14 high risk viruses' specific DNA using polymerase chain reaction (PCR). It contains reagents and enzymes for the specific amplification of the conserved region of the HPV genome, and for the direct detection of the specific amplicon in FAM & HEX channel. In addition, it contains an endogenous control [Human gene] amplification system to identify possible PCR inhibition and DNA purification efficiency. External positive control is supplied, which can be used as both qualitative and quantitative to determination the amount of viral load.

## SPECIFICITY

HPV 14 high risk virus primer and probe have been designed for the specific and exclusive in vitro detection of HPV 14 high risk viruses [HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68]. Thetarget sequence (L6/L7 gene) is highly conserved and has previously been shown to be a good genetic marker for HPV. The primers and probe sequences in this kit have 100% homology with a broad range of clinically relevant reference sequences based on a comprehensive bioinformatics analysis.

## ANALYTICAL SENSITIVITY

The analytical sensitivity is defined as the concentration of DNA molecules (copies/µl) that can be detected with a positivity rate of 95%. The analytical sensitivity was determined by analysis of dilution series of quantified HPV specific DNA from 0.001copies to 10copies/µl in triplicates. Under optimal PCR conditions, the analytical sensitivity is 0.25 copies per micro liter.

## RNA PURIFICATION

If you are using a spin column based sample preparation procedure having washing buffers containing ethanol, it is highly recommended to perform an additional centrifugation step for 5min at approximately 17000 x g (~ 13000 rpm), using a new collection tube, prior to the elution of the DNA.

## ENDOGENOUS CONTROL

Human gene is given as endogenous control. It amplifies a single copy human gene from the test samples. A successful amplification indicates that test samples are properly collected and has its biological property.

The primer and probe present at PCR limiting concentrations which allows multiplexing with the target sequence primers. Amplification of the endogenous control template does not interfere with detection of the mutation even when present at low copy number. The endogenous control is detected through the HEX channel and gives a CT value of 21 +/-10.

## PREPARATION OF STANDARD CURVE DILUTION SERIES:

1. Pipette 90µl of PCR grade water into three 1.5ml micro centrifuge tubes and label as QS2 to QS4.
2. Pipette 10µl of Positive control-QS1 into tube QS2.
3. Vortex thoroughly and spin down briefly.
4. Change pipette tip and pipette 10µl from tube QS2 into tube QS3.
5. Vortex thoroughly and spin down briefly.
6. Repeat steps 4 and 5 to complete the dilution series.
7. Use 10µl per reaction.
8. Prepare fresh every time.

| Standards | Copies per µl |
|---|---|
| QS-1 | 2500000 |
| QS-2 | 250000 |
| QS-3 | 25000 |
| QS-4 | 2500 |

## DETECTION PROTOCOL

- Before use, all kit components need to be thawed completely, mixed by gently inverting and centrifuged briefly.
- Make sure that Positive and Negative control is included in every run.
- Include 0.5 reaction volume for pipetting error while calculating the volume for total number of reactions.

| Components | Volume per reaction | |
|---|---|---|
| | HPV 16 & 18 | HPV 12 & Endo |
| Probe PCR Master Mix | 10µl | 10µl |
| HPV 16 & 18 PP Mix | 5µl | -- |
| HPV 12 PP Mix | -- | 2.5µl |
| Endogenous Mix volume | -- | 2.5µl |
| **Purified DNA** | **10µl** | **10µl** |
| Final reaction volume | 25µl | 25µl |

**Negative Control setup [NTC]**
Add 10µl of PCR grade water.

**Qualitative Positive Control setup**
Add 10µl of any one of the Positive controls [From QS1 to QS4]

**Quantitative Positive controls setup [Suggested to use HPV 16/18 PP mix for Quantitative]**
10µl of all Positive controls prepared from QS1 to QS4.

## PROGRAMMING THERMAL CYCLER

| | |
|---|---|
| Sample volume | 25µl |
| Fluorescence Dyes | FAM & HEX |
| Passive reference | None |
| Ramping rate | Default |

| Cycles | Step | Time | Temp |
|---|---|---|---|
| 1 | Taq enzyme activation / Hold | 15min | 95ºC |
| 45 cycles | Denaturation | 20sec | 95ºC |
| | Annealing/Data collection* | 20sec | 56ºC |
| | Extension | 20sec | 72ºC |

| Data collection/Acquisition | Targets |
|---|---|
| FAM | HPV 16 & HPV 12 |
| HEX | HPV 18 & Endogenous control |

## READING THE GRAPH

**Step-1 – Endogenous control Validation**
Select the test samples alone for the endogenous control analysis. Select HEX dye and view the graph of endogenous control amplification. A successful amplification Ct value must be within Ct 21 +/- 10.

This range indicates NO PCR inhibition in the reaction. Any sample value goes beyond Ct 33 indicates that either sample has some issues in the purification or inhibiting PCR reaction.

Endogenous control will not get amplified in the negative and positive controls. Ignore a late noise HEX amplification graph in the NTC and Positive control wells.

**Step-2 – FAM - Negative and Positive control validation**
Select the NTC and Positive control [Qualitative] or Standards wells [Quantitative], select FAM channel, and view the graph of amplification.

The NTC must be flat with no Ct value. If required adjust the threshold value just above the NTC. The PC or Standards must be amplified as per their copy numbers.

NTC justifies NO contamination in the reagent as well as fine pipetting and its environment. PC justifies the reagents storage conditions and reaction parameters are as prescribed.

**Step-3 –FAM - Test Sample status**
In FAM channel, select test sample well one by one, analyze the graph/amplification.

## QUALITATIVE INTERPRETATION OF RESULTS

| Test Sample | Negative Control | Positive Control | Internal Control | Interpretation |
|---|---|---|---|---|
| Positive | Negative | Positive | Positive | HPV specific DNA detected |
| Negative | Negative | Positive | Positive | No HPV specific DNA Detected. Sample does not contain detectable amounts of HPV specific DNA. |
| Negative | Negative | Negative | Negative | Experiment fail |
| Positive | Positive | Positive | Positive | Experiment fail |

**Calculating copies or IU per ml**

Input the machine indicated copy number into the following formula

$$\text{Result (copies/ml)} = \frac{\text{Result (copies/µl) x Elution Volume (µl)}}{\text{Sample Volume (ml)}}$$

**Note:**

**Elution volume:** must be typed in micro liter format, example 30µl, 60µl or 100µl. **Sample volume:** must be typed in milliliter format, example 0.2ml or 0.5ml

## LIMITATIONS

A false negative result may occur if inadequate numbers of organisms are present in the sample due to improper collection, transport or handling. Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.

The presence of PCR inhibitors may cause under quantification, false negative or invalid results.

The presence of PCR inhibitors may cause under quantification, false negative or invalid results.

Potential mutations within the target regions of the pathogen's genome covered by the primers and/or probes used in the kit may result in under quantification and/or failure to detect.

## QUALITY CONTROL

In accordance with in house Quality Management System, each lot of HPV 14 high risk virus Real-time PCR kit is tested against predetermined specifications to ensure consistent product quality.

### Index of symbols

| | | |
|---|---|---|
| Consult instructions for use | LOT Batch code | Tests per kit |
| IVD For *in vitro* diagnostic use only | Do not re-use | Keep dry |
| 2°C 8°C Temperature limit - 25 °C to - 15 °C | Date of manufacture | Biologial Risks |
| Manufacturer | Use-by date | |

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