Quantiplus® HPV 16 & 18 RT PCR Kit (Real-Time Qualitative PCR Kit)

QL-HPV-25 : 25 rxns
QL-HPV-50 : 50 rxns
QL-HPV-100 : 100 rxns

RUO

PI/QLHPV-01

Intended Use

Quantiplus® HPV 16 & 18 RT PCR Kit is a Real-Time PCR based in vitro diagnostic assay for detection of Human Papilloma Virus (Genotypes 16 and 18) in vaginal swab/cervical brush and tissue samples. The kit consists of HPV 16 and 18 Ready Mix which consists of specific Primers, Probes and Amplification Mix, HPV 16 & 18 Positive Control, and Internal Control (Huwel IC-B mix). The Huwel IC-B mix is a second amplification system used to identify possible PCR inhibition without affecting the analytical sensitivity of the assay.

Background Information

Human Papilloma virus (HPV) is a member of the Papilloma virus family of viruses that is capable of infecting humans. Like all Papilloma viruses, HPVs establish productive infections only in the stratified epithelium of the skin or mucous membranes. HPV infection is the causative agent for almost 90% of all the cervical cancers. HPV types are categorized as low or high-risk based on their oncogenic potential. Low-risk HPV types are typically associated with genital warts, whereas high-risk (HR) types are associated with invasive cervical cancer. Of the HR (oncogenic) HPV types, HPV 16 causes more than 50% of cervical cancers and HPV 18 causes 10% to 20%. Most HPV infections in young females are temporary and have little long-term significance. 70% of infections are gone in 1 year and 90% in 2 years. But when infection persists in 5% to 10% of infected women-there is high risk of developing cervical pre cancer (lesions on the cervix), which can progress to invasive cervical cancer. This process usually takes 15–20 years, providing many opportunities for detection and treatment of the pre-cancerous condition, often with high cure rates.

Kit Components

Color Coding (Caps)	Contents	Description	25 rxns (QL-HPV-25)	50 rxns (QL-HPV-50)	100 rxns (QL-HPV-100)
Amber	HPV 16 & 18 Ready Mix	HPV 16 & 18 and internal control primers, probes and amplification mix	1 x 375 µL	1 x 750 µL	2 x 750 µL
Natural	Huwel IC-B Mix	Internal Control	1 x 300 µL	1 x 600 µL	2 x 600 µL
Red	HPV 16 & 18 PC	HPV 16 & 18 Positive Control	1 x 100 µL	1 x 100 µL	2 x 100 µL
White	Huwel PW	Purified water	1 x 500 µL	1 x 500 µL	2 x 500 µL

Note: Please pay attention to the cap color coding and the tube contents.
Huwel PW (Molecular biology grade purified water)

Storage and Transportation Conditions

The kit should be transported at temperature below −20 °C. The kit is stable until the expiry date mentioned on the package, if the storage temperature is within −20 ±5 °C. More than 4X freezing and thawing cycles reduces the assay sensitivity. For intermittent usage the reagents should be frozen in aliquots.

Technical specifications

Target Sequence	Specific region of the pathogen genome
Sensitivity	10 copies/PCR for HPV 16, 12.5 copies/PCR for HPV 18
Specificity	100%
Reporting units	Detected/Not detected
Validated Specimen	Vaginal Swab/Cervical Brush, Tissue

Assay Procedure

DNA Extraction

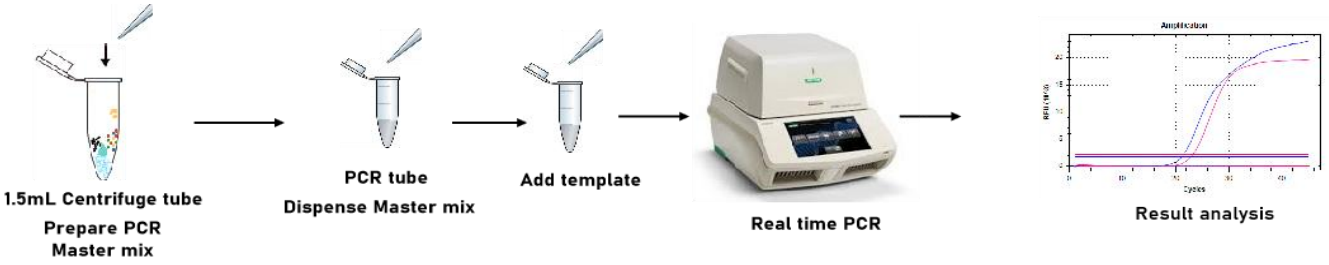
Quantiplus® HPV 16 & 18 RT PCR Kit has been validated using the following DNA extraction kits:

Recommended sample volume for extraction and elution are as follows:

S. No.	Name of the Extraction Kit	Recommended Sample volume for Extraction	Recommended Final Elution volume
1.	Huwel Nucleic Acid Extraction Kit - Version 2.0 (Cat. No. HL-NAX-100)	200 µL	100 µL
2.	QIAamp® DNA Mini Kit (Cat. No. 51304)	200 µL	100 µL

Note: Customer can also validate their own extraction process using other DNA extraction Kits.
IC-B mix can be added at the extraction step or while setting up the PCR

qPCR Protocol Flow



Preparation of Reaction Master mix

Components	Volume per reaction (for 26µL)
HPV 16 & 18 Ready Mix	15.0
Huwel IC-B Mix	1.0
Extracted DNA/ HPV 16 & 18 PC / Huwel PW	10.0

It is necessary to keep all components at +2 °C to +8 °C during the PCR preparation. Close the tubes and centrifuge briefly before proceeding to the thermal cycler.

Cycling Conditions

Steps	No. of cycles	Temperature (°C)	Time
1 (Initial denaturation)	1	95	15 min.
2 (PCR cycling)	40	90	30 sec.
		56	30 sec.
		60*	30 Sec
* **Plate Read/Data Acquisition in FAM, TEXAS RED/ ROX and Yakima Yellow /VIC/HEX channel**			

Sample analysis and Interpretation

The criteria for the acceptance of the assay should be met before the interpretation of the unknown sample results as described in Table 1 below. Interpret the results for unknown samples as mentioned in Table 2.

Table 1

Control	FAM (HPV16)	TEXAS RED/ ROX (HPV 18)	Yakima Yellow/ HEX/ VIC (IC)
If Internal Control (Huwel IC-B Mix) is added during extraction			
Positive Control (PC)	√	√	-
Negative Control (NC)	-	-	-
If Internal Control (Huwel IC-B Mix) is added during preparation of reaction master mix			
Positive Control (PC)	√	√	√
Negative Control (NC)	-	-	√

Table 2

S.No	FAM (HPV16)	TEXAS RED/ ROX (HPV 18)	Yakima Yellow/ HEX/ VIC (IC)	← Fluorophore / Interpretation ↓	Conclusion
1	√	√	√	HPV (16 &18) DNA detected	Proceed for further Analysis
2	√	√	-	HPV (16&18) DNA detected	
3	√	-	√	HPV 16 DNA detected	
4	-	√	√	HPV 18 DNA detected	
5	-	-	√	HPV (16 & 18) DNA not detected	
6	-	-	-	Possible inhibition of PCR	Dilute the DNA sample (1:10) and repeat the assay

Note: All the Target channels (FAM, Texas Red/ROX and Yakima Yellow/ VIC/HEX) to be analyzed individually.

Validated Instruments

- Thermo QS5 Real-Time PCR System
- Bio-Rad™ CFX 96

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