

## Quantiplus® HSV 1&2 Quantitation Kit (Real-Time Quantitative PCR Kit)



QT-HSV-25 : 25 rxns  
 QT-HSV-50 : 50 rxns  
 QT-HSV-100 : 100 rxns



PI/QTHSV-01

### Intended Use

Quantiplus® HSV 1 & 2 Quantitation Kit (Real-Time Quantitative PCR Kit) is an in-vitro diagnostic kit for detection and quantitation of Herpes Simplex Virus in human plasma, ocular fluid and cerebrospinal fluid (CSF). The kit contains necessary reagents for performing HSV 1 & 2 quantitation using Real-Time PCR. The assay is based on the amplification of specific region (Glycoprotein D) of the virus. The kit contains single tube qPCR mix with Primers and Probe Mix (PPM) for HSV 1 & 2, Internal Control; HSV 1 & 2 Standards (HSVQS1-HSVQS4), Internal Control (Huwel IC-B mix). The internal control mix (IC-B Mix) helps to identify possible PCR inhibition without affecting the analytical sensitivity of the assay.

### Background Information

Herpes simplex is a viral disease caused by both Herpes Simplex Virus 1 (HSV-1) and Herpes Simplex Virus 2 (HSV-2). As per estimates, in 2016, 3.7 billion and 500 million people worldwide are seropositive for HSV1 and HSV2, respectively. Herpes simplex is most easily transmitted by direct contact with a lesion or body fluids of an infected individual. HSV symptoms can range from asymptomatic, mild to life threatening. In few cases HSV infection is also associated with high morbidity and mortality for reasons that are unknown. HSV causes cold sores, genital herpes, meningitis and herpes simplex encephalitis (HSE). HSV infection is also linked to neurodegenerative diseases. Laboratory testing is often used to confirm the diagnosis. Laboratory tests include: culture of the virus, direct fluorescent antibody (DFA) studies for detection, skin biopsy, and polymerase chain reaction (PCR) to test for presence of viral DNA.

### Kit Components

Color Coding (Caps)	Contents	Description	25 rxns (QT-HSV-25)	50 rxns (QT-HSV-50)	100 rxns (QT-HSV-100)
Amber	HSV Ready Mix	Primer and probes, for HSV 1& 2, Internal Control, 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
Pink	HSVQS1	2X10 <sup>4</sup> copies/µL	1 x 100 µL	1 x 100 µL	2 x 100 µL
Pink	HSVQS2	2X10 <sup>3</sup> copies/µL	1 x 100 µL	1 x 100 µL	2 x 100 µL
Pink	HSVQS3	2X10 <sup>2</sup> copies/µL	1 x 100 µL	1 x 100 µL	2 x 100 µL
Pink	HSVQS4	2X10 <sup>1</sup> copies/µL	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 temperatures 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 reduce the assay sensitivity. For intermittent usage the reagents should be frozen in aliquots.

**Technical specifications**

Target Sequence	Glycoprotein D gene
Specificity	100%
Sensitivity	1 copy/ $\mu$ L and 500 copies /mL
Linear range	4 copies / $\mu$ L to $2 \times 10^7$ copies/ $\mu$ L
Validated Specimen	Plasma (K2EDTA-Blood), CSF, and Ocular fluid

**Assay Procedure**

**DNA Extraction**

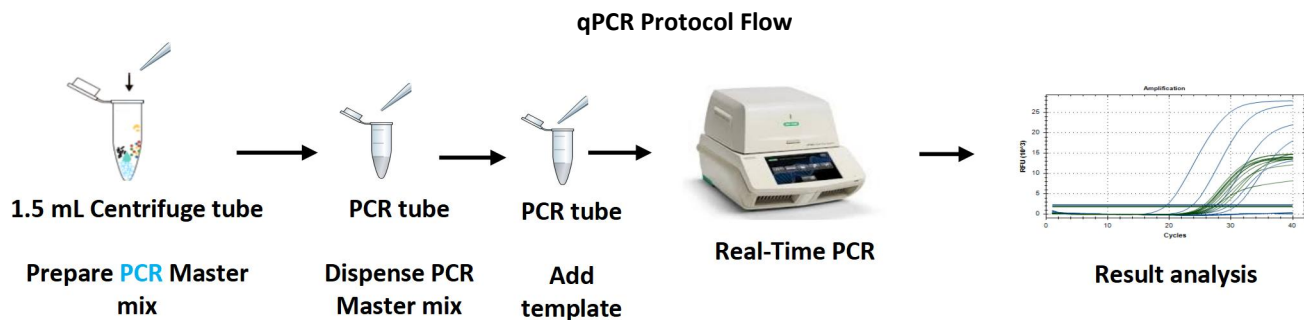
Quantiplus® HSV 1 & 2 Quantitation Kit (Real-Time Quantitative PCR Kit) has been validated using the following DNA extraction kits. Recommended sample volume for extraction and elution are as follows:

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 $\mu$ L	100 $\mu$ L
2.	QIAamp® DNA Blood Mini Kit (Cat. No. 51104)	200 $\mu$ L	100 $\mu$ L

*Note: Customer can also validate their own extraction process using other Viral DNA extraction Kits.*

*IC-B mix can be added at the extraction step or while setting up the PCR*

*The recommended sample volumes for extraction and elution are also applicable for CSF and ocular fluid sample types.*



**Preparation of Reaction Master mix**

Components	Volume per reaction (for 26 $\mu$ L)
HSV Ready Mix	15.0
Huwel IC-B Mix (If not added during extraction)	1.0
Extracted DNA/ HSVQS1- HSVQS4 / 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	95	15 sec.
		60*	1 min.

\* Plate Read/Data Acquisition in **FAM** and **Yakima Yellow /VIC/HEX** channel

**Sample analysis and Interpretation**

Interpret the values for unknown samples, only if the Slope of Standards is between -3.1 to -3.6 (at least 3 standards should be included) and PCR efficiency is between 90%-110% (0.9 – 1.1) and there should be no amplification in the FAM channel for negative control.

S.No	FAM (HSV)	Yakima Yellow / VIC /HEX (IC)	← Fluorophore	
			Interpretation	Conclusion
1	✓	✓	HSV 1 & 2 DNA detected within quantitation range	Proceed for further Analysis
2	✓	-		
3	-	✓	HSV 1 & 2 DNA below quantitation limit	Dilute the DNA sample (1:100) and repeat the Assay
4	-	-	Possible inhibition of PCR	

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

**i** To convert the results of copies/μL obtained using the standard curve to copies/mL of the sample use the formula mentioned below.

$$\text{Result (copies/mL)} = \frac{\text{Result (copies/}\mu\text{L)} \times \text{Elution Volume (}\mu\text{L)}}{\text{Sample Volume (mL)}}$$

**i** \* For calculating the result of diluted sample (1:100); multiply the observed copies/mL value by dilution factor, 100

$$\text{Result of 1:100 diluted sample (copies/mL)} = \text{Dilution Factor (100)} \times \frac{\text{Result (copies/}\mu\text{L)} \times \text{Elution Volume (}\mu\text{L)}}{\text{Sample Volume (mL)}}$$

**Validated Instruments**

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



HLSS Manufacturing Pvt Ltd  
 Plot No's M14, M15, M16, TSIIC Medical device park  
 Sultanpur village, Ameenpur Mandal,  
 SangareddyDist, TS-502319