Quantiplus® Epstein Barr Virus Real-Time Quantitative PCR Kit

QT-EBV-25 : 25 rxns
QT-EBV-50 : 50 rxns
QT-EBV-100 : 100 rxns

IVD

PI/QTEBV-03

Intended Use

Quantiplus® Epstein Barr Virus Real-Time Quantitative PCR Kit is a Real-Time PCR based in vitro diagnostic assay for detection and quantitation of Epstein Barr Virus in human plasma. The kit contains Amplification Mix with specific Primers and Probes, Standards (EBQS1 – EBQS4) and Internal Control (IC-B). The IC-B helps to identify possible PCR inhibition without affecting the analytical sensitivity of the assay.

Background Information

Epstein-Barr virus (EBV), also known as Human Herpes Virus 4, is a member of the Herpes Virus family. It is one of the most common human viruses. EBV is found all over the world. Most people get infected with EBV at some point in their lives. EBV spreads most commonly through body fluids, primarily saliva. EBV can cause infectious mononucleosis. Many people become infected with EBV in childhood. EBV infections in children usually do not cause symptoms, or the symptoms are not distinguishable from other mild, brief childhood illnesses. After an EBV infection, the virus becomes latent in the body. In some cases, the virus may reactivate. This does not always cause symptoms, but people with weakened immune systems are more likely to develop symptoms if EBV reactivates. EBV-DNA in the plasma is diagnosis of primary EBV infection. Since serum and plasma are readily obtained, they may be better sources for the quantitation of the viral load.

Kit Components

Color Coding (Caps)	Contents	Description	25 rxns (QT-EBV-25)	50 rxns (QT-EBV-50)	100 rxns (QT-EBV-100)
Amber	Huwel EBV Ready Mix	Primers and Probes for EBV and Internal Control 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
Pink	Huwel EBQS1	2 X 10^6 IU/µL	1 x 100 µL	1 x 100 µL	2 x 100 µL
Pink	Huwel EBQS2	2 X 10^5 IU/µL	1 x 100 µL	1 x 100 µL	2 x 100 µL
Pink	Huwel EBQS3	2 X 10^4 IU/µL	1 x 100 µL	1 x 100 µL	2 x 100 µL
Pink	Huwel EBQS4	2 X 10^3 IU/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	1 x 1 mL

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 kits should be transported at temperature below −20 °C. The kit is stable until the expiry date printed 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	Specific region in EBNA-1 of EBV Genome
Specificity	100%
Sensitivity	0.2 IU/µl (100 IU/mL)
Linear Range	2 x 10^6 – 0.2 IU/µL (1 x 10^9 1 to 10^3 IU/mL)

Reporting Units	IU/µl (1 IU = 1.9 copies/µL)
Validated Specimen	Plasma
External Quality Assessment	QCMD EQA Panels

Assay Procedure

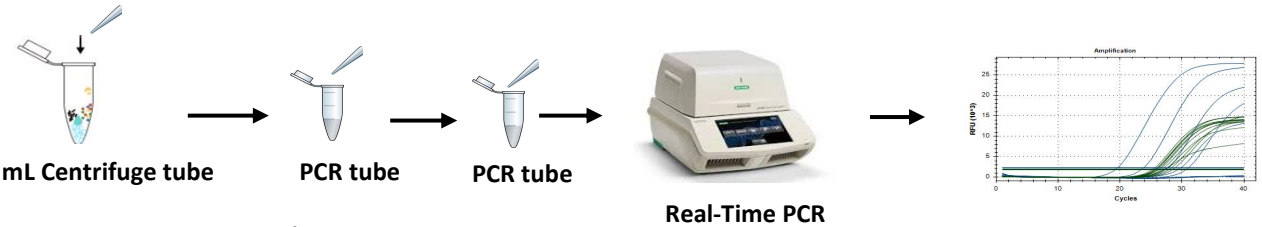
DNA Extraction

Quantiplus® Epstein Barr Virus Real-Time Quantitative PCR Kit has been validated using the following Viral DNA extraction kits. Recommended sample volumes 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 Blood Mini Kit (Cat. No. 51104)	200 µL	100 µL

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

qPCR Protocol Flow



Preparation of Reaction Master Mix

Components	Volume per reaction (for 26µL)
Huwel EBV Ready Mix	15.0
Huwel IC-B Mix (if not added at extraction step)	1.0
Extracted DNA// Huwel EBQS1-Huwel EBQS4/ 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)	45	95	15 sec.
		60*	1 min
***Plate read/Data acquisition in FAM 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 and also the slope of the standard curve (standards in FAM channel) is between –3.1 to –3.6, (at least three standards should be included), and PCR efficiency is between 90% to 110% (0.9 to 1.1). Interpret the results of unknown samples as mentioned in Table 2

Table 1:

Control	FAM (EBV)	YAKIMA YELLOW/ VIC/HEX (IC)
If Internal Control (IC-B Mix) is added during extraction		
Standards (EBQS1 to EBQS4)	√	-
Negative Control (NC)	-	-
If Internal Control (IC-B Mix) is added during preparation of reaction master mix		
Standards (EBQS1 to EBQS4)	√	√
Negative Control (NC)	-	√

Table 2:

S.No	FAM (EBV)	YAKIMA YELLOW/ VIC/HEX (IC)	Fluorophore / Interpretation	Conclusion
1	√	√	EBV DNA detected within the quantitation range	Proceed for further Analysis
2	√	-		
3	-	√	EBV DNA below quantitation limit	
4	-	-	Possible inhibition of PCR	Dilute the DNA sample (1:10) and repeat the Assay

Note: All the Target channels (FAM, and YAKIMA YELLOW/ VIC/ HEX) to be analyzed individually.

Viral load calculation (Conversion of IU/µL to IU/mL)

$$\text{IU/mL} = \frac{\text{Obtained IU/µL X Elution Volume (µL)}}{\text{Sample volume in mL}}$$

For calculating the result of diluted sample (1:10); multiply the observed IU/mL value by dilution factor, 10

$$\text{Result of 1:10 diluted sample (IU/mL)} = \text{Dilution Factor (10)} \times \frac{\text{Result (IU/µL) x Elution Volume (µL)}}{\text{Sample Volume (mL)}}$$

Reporting Comments

Results in IU/mL	Comments
Target not detected	EBV DNA not detected in the given sample
<100	EBV DNA detected but below the lower limit of the quantitation range of the assay. The reproducibility of the positive result is not assured.
1×10^3 to 1×10^9	EBV DNA detected within the linear range of the assay.
$> 1 \times 10^9$	EBV DNA detected but above linear range of the assay, dilute the sample for accurate result.

Validated Instruments

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

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