Quantiplus® BK Virus Real-Time Quantitative PCR Kit

QT-BKV-25 : 25 rxns
QT-BKV-50 : 50 rxns
QT-BKV-100 : 100 rxns

IVD

PI/QTBKV-01

Introduction

The BK polyoma virus (BKV) is a member of the *polyomaviride* family of double stranded DNA (dsDNA) viruses. It is the etiologic agent of Progressive Multifocal Leukoencephalopathy (PML) and was first isolated in 1971 from a urine sample obtained from a renal transplant recipient. Diagnosis is advised to renal transplant patient with deterioration in renal function and transplant patient presenting with hemorrhagic cystitis.

Product Description

Quantiplus® BK Virus Real-Time Quantitative PCR Kit is a Real Time PCR based *in vitro* diagnostic assay for quantitation of BK Virus in human plasma and Urine. The kit contains Amplification Mix with specific Primers and Probes, Standards (BKQS1-BKQS4), and Internal Control.

Kit components

Color Coding (Caps)	Contents	Description	25 rxns (QT-BKV-25)	50 rxns (QT-BKV-50)	100 rxns (QT-BKV-100)
Amber	Huwel BK Ready Mix	Probes and Primers for BK virus and Internal Control along with Amplification Mix	1 x 375 µL	1 x 750 µL	2 x 750 µL
Natural	Huwel IC-A Mix	Internal Control	1 x 300 µL	1 x 600 µL	2 x 600 µL
Pink	Huwel BKQS1	2 X 10^6 IU/µL	1 x 50 µL	1 x 100 µL	1 x 200 µL
Pink	Huwel BKQS2	2 X 10^5 IU/µL	1 x 50 µL	1 x 100 µL	1 x 200 µL
Pink	Huwel BKQS3	2 X 10^4 IU/µL	1 x 50 µL	1 x 100 µL	1 x 200 µL
Pink	Huwel BKQS4	2 X 10^3 IU/µL	1 x 50 µL	1 x 100 µL	1 x 200 µL
White	Huwel PW	Purified Water	1 x 500 µL	1 x 500 µL	1 x 1 mL

Storage and Transportation Conditions

The kits could be transported at temperature below -20 °C. The kit will remain stable until the expiry date printed on the package, if the storage temperature is kept (-20 ± 5 °C). Kit is stable after 4 repeated freezing/thawing cycles. The reagents should be frozen in aliquots, if they are to be used intermittently.

Technical Specification

Target Sequence	Conserved region of *VP1* gene
Specificity	100%
Sensitivity	0.2 IU/µL (100 IU/mL or 100 copies/mL)
Linear Range	2 x 10^7 – 0.2 IU/µL (1 x 10^{10} – 1 x 10^2 IU/mL or 1 x 10^{10} – 1 x 10^2 copies/mL)
Reporting Units	IU/mL or Copies/mL (Conversion factor: 1 IU = 1 copy)
Validated Specimen	Plasma
External Quality Assessment	QCMD EQA Panels

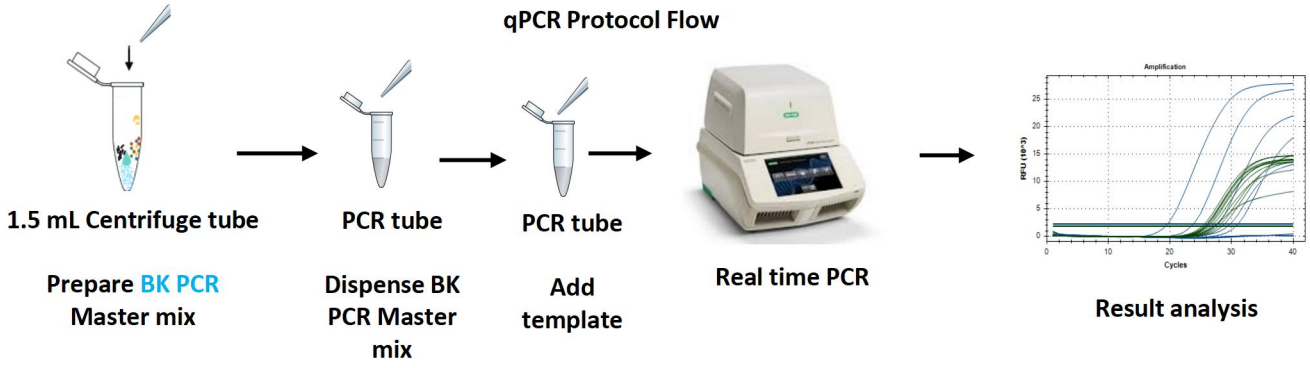
Assay Procedure

DNA Extraction

Quantiplus® BK Virus Real-Time Quantitative PCR Kit has been validated using the following Viral 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 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.



Preparation of Reaction Master mix

Components	Volume per reaction (for 26 µL)
Huwel BK Ready Mix	15.0
Huwel IC-A Mix (if not added at extraction step)	1.0
Extracted DNA/Huwel BKQS1- Huwel BKQS4/ 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 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 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 and PCR efficiency is between 90%-110% (0.9 - 1.1) and there should be no amplification in negative control.

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

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

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

Validated Instruments

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

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