Quantiplus® JE Virus Detection Kit (Real-Time Qualitative PCR Kit)

QL-JEV-25 : 25 rxns
QL-JEV-50 : 50 rxns
QL-JEV-100 : 100 rxns

RUO

PI/QLJEV-01

Intended Use

Quantiplus® JE Virus Detection Kit is a Real-Time PCR based in vitro diagnostic assay for detection of JE Virus in human plasma and cerebrospinal fluid. The kit consists of primers and probes for JEV, Internal Control , JEV 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

Japanese Encephalitis Virus (JEV) is an enveloped positive single stranded RNA virus belonging to genus Flavivirus in the family Flaviviridae. It is the most common agent of viral encephalitis, causing an estimated 50,000 cases annually, of which 15,000 will die and up to 50% of survivors are left with severe residual neurological complications. The first step in virus infection requires interaction between the virus attachment proteins (VAP) and cellular receptors. The interaction of VAP and its cellular receptors is known to contribute to host range, tissue tropism and viral pathogenesis.

Kit Components

Color Coding (Caps)	Contents	Description	25 rxns (QL-JEV-25)	50 rxns (QL-JEV-50)	100 rxns (QL-JEV-100)
Green	R Core qPCR Mix (2X)	Amplification Reagent	1 x 325 µL	1 x 650 µL	2 x 650 µL
Amber	JEV Primer Probe Mix	JEV, Internal Control Primers and Probes Mix	1 x 50 µL	1 x 100 µL	2 x 100 µL
Natural	Huwel IC-B Mix	Internal Control	1 X 300 µL	1 X 600 µL	2 X 600 µL
Red	JEV Positive Control	JEV 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 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	Envelope protein gene
Specificity	100%
Sensitivity	10 copies/PCR
Validated Specimen	Plasma (K2EDTA-Blood)

Assay Procedure

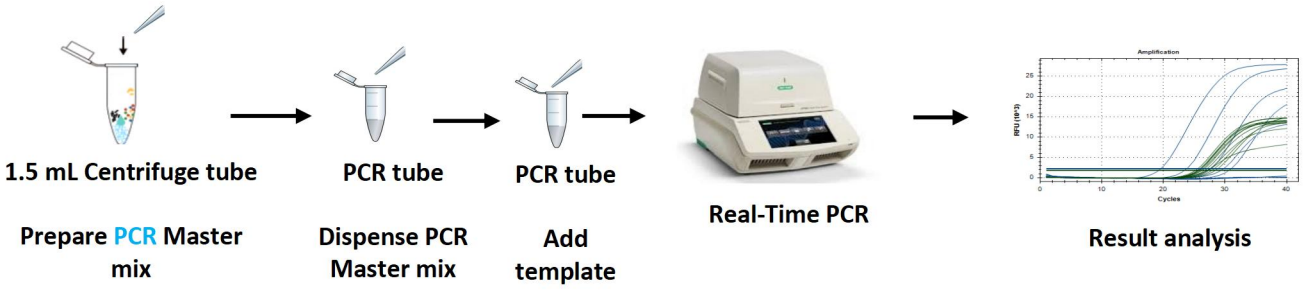
RNA Extraction

Quantiplus® JE Virus Detection Kit (Real-Time Qualitative PCR Kit) has been validated using the following Viral RNA 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®Viral RNA Mini Kit (Cat. No. 52904)	140 µL	50 µL

Note: Customer can also validate their own extraction process using other Viral RNA 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 sample types.

qPCR Protocol Flow



Preparation of Reaction Master mix

Components	Volume per reaction (for 26µL)
R Core qPCR Mix (2X)	13.0
JEV Primer Probe Mix	2.0
Huwel IC-B Mix	1.0
Extracted RNA/ JEV Positive Control /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 (Reverse Transcription)	1	42	15min
2 (Initial denaturation)	1	95	15 min.
3 (PCR cycling)	40	95	15 sec.
		60*	1 min.
***Plate read/Data acquisition in FAM and Yakima Yellow/ VIC/HEX channels**			

Sample analysis and Interpretation

For unknown sample analysis the cutoff Ct for JEV RNA (FAM) and IC (YAKIMA YELLOW/ HEX/ VIC) are ≤36 and ≤32 respectively. 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 (JE Virus)	Yakima Yellow/VIC/HEX (Internal Control)
If Internal Control (IC-B Mix) is added during extraction		
Positive Control (PC)	√	-
Negative Control (NC)	-	-
If Internal Control (IC-B Mix) is added during preparation of reaction master mix		
Positive Control (PC)	√	√
Negative Control (NC)	-	√

Table 2

S.No	FAM (JEV)	Yakima Yellow/ VIC/HEX (IC)	Fluorophore / Interpretation	Conclusion
1	√	√	JEV RNA detected	Proceed for further Analysis
2	√	-		
3	-	√	JEV RNA not detected	
4	-	-	Possible inhibition of PCR	Dilute the RNA sample (1:10) and repeat the Assay

Note: All the Target channels (FAM 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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