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

QL-ZKV-25 : 25 rxns
QL-ZKV-50 : 50 rxns
QL-ZKV-100 : 100 rxns

RUO

PI/QLZKV-01

Intended Use

Quantiplus® Zika virus Detection kit is used to detect Zika virus in plasma and urine samples. The kit contains qPCR Mix, Primer probe Mix (PPM) along with Zika Virus positive control (Zika PC) and Internal control (IC-B Mix).

Background Information

Zika virus is carried by infected Aedes mosquitos (*Ae. aegypti and Ae. albopictus*), Zika is largely transmitted through bite of an infected mosquito. Presumptive sexual transmission to women from partners suffering from Zika infection has also been reported. In infected pregnant women, Zika can cross the placenta and affect the fetus. While anyone can contract Zika, pregnant women are most at risk due to fetal abnormality and other neurological abnormalities. The Quantiplus® Zika virus Detection Kit is used to detect Zika Virus in human plasma and urine.

Kit Components

Color Coding (Caps)	Contents	Description	25 rxns (QL-ZKV-25)	50 rxns (QL-ZKV-50)	100 rxns (QL-ZKV-100)
Green	R Fast Core qPCR Mix (2X) with ROX	PCR Amplification Mix along with cDNA synthesis reagent	1 x 325 µL	1 x 650 µL	2 x 650 µL
Amber	Zika PPM	Zika Virus and internal control Primer Probe Mix	1 X 50 µL	1 X 100 µL	2 X 100 µL
Natural	IC-B Mix	Internal Control	1 X 300 µL	1 X 600 µL	2 X 600 µL
Red	Zika PC	Zika Positive Control	1 x 100 µL	1 X 100 µL	2 X 100 µL
White	MBGPW	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.
MBGPW (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

Specificity	100%
Reporting units	Detected/Not detected
Validated Specimen	Plasma (K2EDTA-Blood) and Urine

Assay Procedure

RNA Extraction

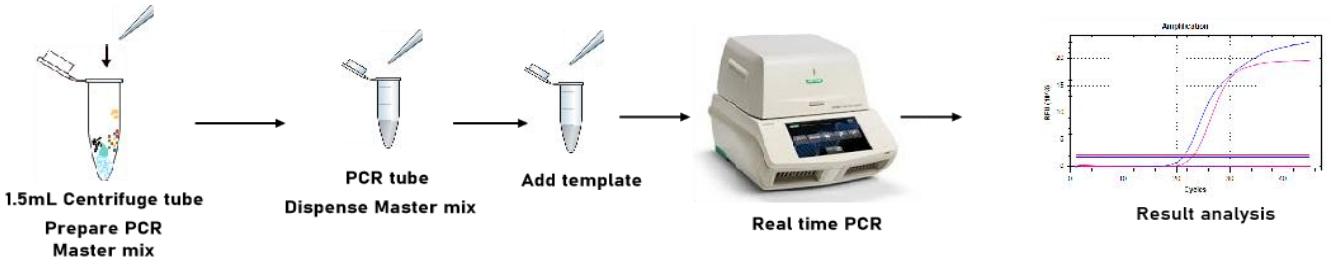
Quantiplus® Zika Virus Detection Kit has been validated using the following RNA extraction kits:

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	60 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

qPCR Protocol Flow



Preparation of Reaction Master mix

Components	Volume per reaction (for 26µL)
R Fast core qPCR Mix (2X) with ROX	13.0
Zika PPM	2.0
IC-B Mix (if not added at extraction step)	1.0
Extracted RNA/ Zika PC/ MBGPW	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	53	5 min
1 (Initial denaturation)	1	95	3 min.
2 (PCR cycling-1)	45	95	15 sec.
		60	30 sec.
* 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. Interpret the results for unknown samples as mentioned in Table 2.

Table 1

Control	FAM (Zika)	Yakima Yellow / VIC /HEX (IC)
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 (ZIKA)	Yakima Yellow / VIC /HEX (IC)	Fluorophore ← / Interpretation ↓	Conclusion
1	√	√	Zika RNA detected	Proceed for further Analysis
2	√	-	Zika RNA detected	
3	-	√	Zika 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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