Quantiplus® Dengue Virus Real-Time Qualitative PCR Kit

QL-DEN-25 : 25 rxns
QL-DEN-50 : 50 rxns
QL-DEN-100 : 100 rxns

IVD

PI/QLDEN-02

Intended Use

Quantiplus® Dengue Virus Real-Time Qualitative PCR Kit is a Real-Time PCR based in vitro diagnostic assay for the detection of Dengue virus in human plasma. The kit contains Amplification Mix with Primer Probe Mix, Positive Control and Internal Control (IC-B Mix). The internal control mix helps to identify possible PCR inhibition without affecting the analytical sensitivity of the assay.

Background Information

Dengue virus is a member of the family Flaviviridae with a single stranded positive sense RNA genome. The virus particle consists of a lipid bilayer with envelope (E) and membrane (M) proteins surrounding a spherical nucleocapsid composed of an RNA genome and capsid (C) proteins. The virion structure is easily destroyed by treatment with nonionic detergent or low osmotic shock. Four antigenically related but distinct serotypes of dengue virus (DEN1, DEN2, DEN3, and DEN4) are circulating in tropical countries and cause dengue fever and dengue hemorrhagic fever, both of which are highly prevalent.

Kit components

Color Coding (Caps)	Contents	Description	25 rxns (QL- DEN-25)	50 rxns (QL-DEN-50)	100 rxns (QL-DEN-100)
Green	R FastCore qPCR mix (2x) with ROX	Amplification Mix along with cDNA synthesis reagent	1 x 325 µL	1 x 650 µL	2 x 650 µL
Amber	Dengue PPM	Probes and Primers mix forDengue virus and Internal Control	1 x 50µL	1 x 100 µL	2 x 100 µL
Red	Huwel Dengue PC	Dengue Positive Control	1 x 100 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
White	MBGPW	Purified Water	1 x 500 µL	1 x 500 µL	1 x 1mL

Note: Please pay attention to the cap color coding and the tube contents.
MBGPW: 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 reduces the assay sensitivity. For intermittent usage the reagents should be frozen in aliquots.

Technical Specification

Target Sequence	Specific region in *NS1* gene of Dengue virus
Specificity	100%
Limit of Detection	≥10 RNA copies/10 µL of sample.
Validated Specimen	Plasma

Assay Procedure

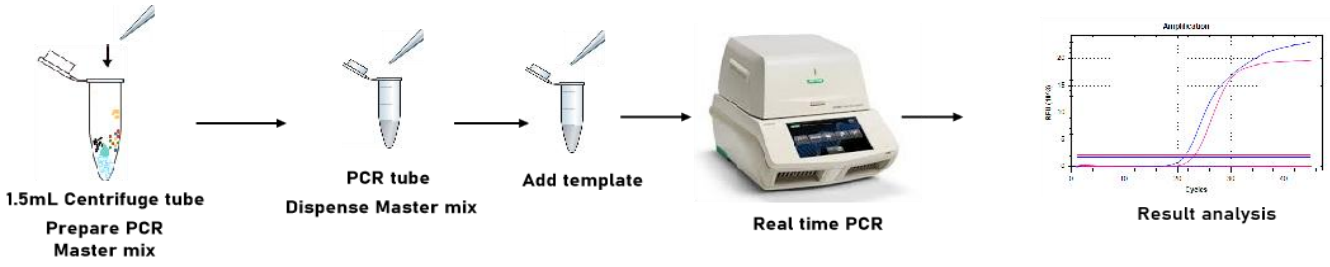
RNA Extraction

Quantiplus® Dengue Virus 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
1.	QIAamp® Viral RNA Mini Kit (Cat. No. 52904)	140 µL	60 µL

Note: Customer can also validate their own extraction process using other Viral Nucleic acid extraction Kits.

qPCR Protocol Flow



Preparation of Reaction Master mix

Components	Volume per reaction (For 26 µL)
R Fastcore qPCR mix(2x) with ROX	13.0
Dengue PPM	2.0
Huwel IC-B Mix (if not added during extraction)	1.0
Extracted RNA/ Huwel Dengue PC / MBGPW	10.0

***Note: Set the reaction volume as 25 µL (the final volume is 26.0 µL but selecting 25 µL does not alter the final result or assay sensitivity).**

It is necessary to keep all components at +2 °C to +8 °C during the PCR preparation. Close the tubes, centrifuge briefly before proceeding to thermal cycler.

Cycling Conditions

No. of Steps	Temperature (°C)	Time
1 (Reverse Transcription)	53	5 min
1 (Initial denaturation)	95	03 min
45 (PCR cycling)	95	15 sec
	*58	45 sec
* Plate Read/Data Acquisition in **FAM** and **Yakima Yellow/ VIC/ HEX** channel		

Sample analysis and Interpretation

For unknown sample analysis the cutoff Ct for Dengue RNA (FAM) and IC (YAKIMA YELLOW/ HEX/ VIC) are ≤40 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 (Dengue)	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 (Dengue)	Yakima Yellow/ VIC/HEX (IC)	Fluorophore / Interpretation	Conclusion
1	√	√	Dengue RNA Detected	Proceed for further analysis
2	√	-		
3	-	√	Dengue 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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