Quantiplus® Dengue & Chikungunya Multiplex Detection Kit (Real-Time Qualitative PCR Kit)

QL-MDC-25 : 25 rxns
QL-MDC-50 : 50 rxns
QL-MDC-100 : 100 rxns

RUO

PI/QL-MDC-01

Intended Use

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

Background Information

Chikungunya and Dengue viruses are transmitted to humans through the bite of virus-carrying mosquito, *Aedes aegypti.* Both of these viruses cocirculate and can be transmitted together. Dengue virus is a member of the family *Flaviviridae* with a single stranded positive sense RNA genome. 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. Chikungunya disease is caused by Chikungunya virus, which belongs to the family *Togaviridae*. Chikungunya has a similar clinical presentation as Dengue. Accurate and timely diagnosis of these infections and confirming coinfection if exists will lead to better clinical treatment outcomes.

The real-time PCR, identification possesses several advantages over conventional immunological procedures; including quick detection turnaround time, a lower contamination rate, a higher sensitivity, specificity, and easy standardization.

Kit Components

Color Coding (Caps)	Contents	Description	25 rxns (QL-MDC-25)	50 rxns (QL-MDC-50)	100 rxns (QL-MDC-100)
Green	Rcore qPCR mix (2X)	Amplification Mix	1 x 325 µL	1 x 650 µL	2 x 650 µL
Amber	MDC PPM	Primers and probes mix	1 x 50 µL	1 x 100 µL	2 x 100µL
Red	MDC PC	Dengue & Chikungunya 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
Natural	qPCR Additive	PCR Reaction enhancer	1 x 200 µL	1 x 200 µL	2 x 200 µL
White	Huwel PW	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.
Huwel PW is 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

Pathogen	Dengue	Chikungunya
Target Sequence	Specific region in *NS1* gene of Dengue virus	Specific region in *NS1* gene of Chikungunya virus
Specificity	100%	100%
Limit of detection	10 RNA copies per reaction	10 RNA copies per reaction
Validated Specimen	Plasma	Plasma

Assay Procedure

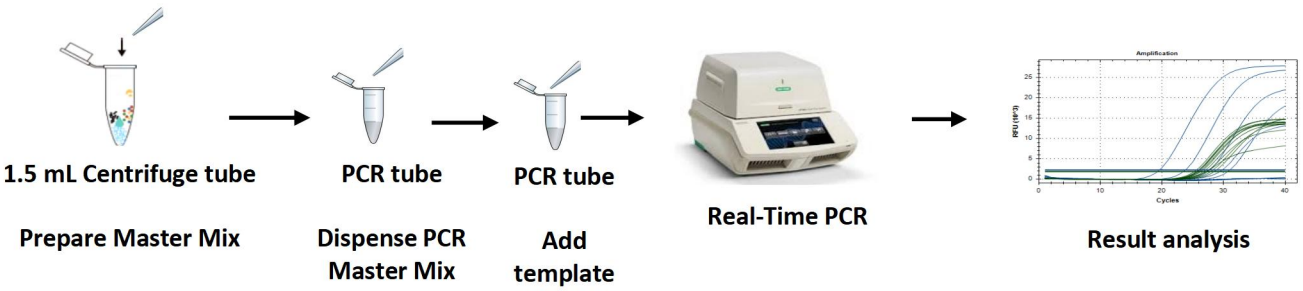
RNA Extraction

Quantiplus® Dengue & Chikungunya Virus multiplex Detection kit (Real-Time Qualitative PCR Kit) has been validated using the Viral RNA extraction kits mentioned below. 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)	200 µL	100 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 27µL)
R core qPCR mix (2X)	13.0
MDC PPM	2.0
qPCR Additive	1.0
Huwel IC-B Mix (if not added during extraction)	1.0
Extracted RNA/ MDC PC /Huwel PW	10.0

Note: Total reaction volume is 27 µL (see above). Performance of the assay is not altered even if the reaction is set to 25 µL (15 µL of Master mix + 10 µL of Template).

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

Step	No. of Cycles	Temperature (°C)	Time
Reverse Transcription	1	53	5 min
Initial denaturation	1	95	15 min
PCR cycling	10	90	30 sec
		54	30 sec
		60	30 sec
PCR cycling	35	90	30 sec
		56	30 sec
		60*	1 min
* **Plate Read/Data Acquisition in FAM and Yakima Yellow / HEX/ VIC , and TEXAS RED channel**			

Note: Consider the first 10 cycles also in the Ct determination. Total number of PCR cycles in the program = 10+35=45.

Results and Interpretation

Interpret the values for unknown samples based on the observations as described in the following table and there should be no amplification in negative control**. The Ct values of ≤ 40 Ct for Dengue and Chikungunya RNA and ≤30 Ct for IC of unknown samples should be considered for positive sample interpretation.**

FAM (Dengue)	YAKIMA YELLOW/HEX/ VIC (Chikungunya)	Texas Red (IC)	Fluorophore / Interpretation	Conclusion
√	√	√	Dengue and Chikungunya RNA Detected	Proceed for further analysis
√	-	√	Dengue RNA Detected	
-	√	√	Chikungunya RNA Detected	
-	-	√	Dengue / Chikungunya RNA Not Detected	
-	-	-	Possible inhibition of PCR	Dilute the RNA sample (1:10) and repeat the Assay

Validated Instruments

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

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