Quantiplus® Chikungunya Real-Time Qualitative PCR Kit

QL-CHK-25 : 25 rxns
QL-CHK-25 : 50 rxns
QL-CHK-100 : 100 rxns

IVD

PI/QLCHK-02

Intended Use

Quantiplus® Chikungunya Real-Time Qualitative PCR Kit is a Real-Time PCR based in vitro diagnostic assay for the detection of Chikungunya virus in human plasma. Chikungunya infection, is detected by amplification of Chikungunya RNA in human plasma. The kit contains Amplification mix with specific Primers and Probes, Positive Control, Internal Control (IC-B Mix). The test results are obtained within 80 minutes unlike other serological tests.

Background Information

Chikungunya disease is caused by Chikungunya virus which belongs to the family Togaviridae that is transmitted to humans by the bite of virus carrying Aedes aegypti mosquito. Chikungunya infections can be diagnosed by common laboratory tests which include virus isolation, serological tests and Reverse Transcriptase Real-Time Polymerase Chain Reaction (RT-PCR).

Kit components

Color Coding (Caps)	Contents	Description	25 rxns (QL-CHK-25)	50 rxns (QL-CHK-50)	100 rxns (QL-CHK-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	Chikungunya PPM	Primers and Probes mix of Chikungunya and Internal control	1 x 50 µL	1 x 100 µL	2 x 100 µL
Red	Huwel Chikungunya PC	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
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 reduce the assay sensitivity. For intermittent usage the reagents should be frozen in aliquots.

Technical Specification

Target Sequence	*NS1* of Chikungunya genome
Specificity	100%
Limit of Detection	10 copies per reaction
Validated Specimen	Plasma

Assay Procedure

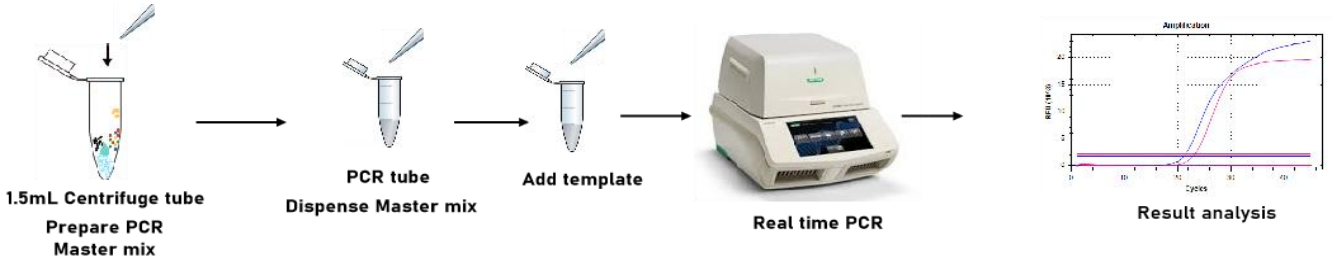
RNA Extraction

Quantiplus® Chikungunya 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)	200 µL	100 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 q PCR Mix(2x) with ROX	13.0
Chikungunya PPM	2.0
Huwel IC-B Mix (if not added during extraction)	1.0
Extracted RNA/ Huwel Chikungunya PC /MBGPW	10.0

Note: Total reaction volume is 26 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 and centrifuge briefly before proceeding to thermal cycler.

Cycling Conditions

No. of cycles	Temperature (°C)	Time
1 (Reverse Transcription)	53	5 min.
1 (Initial Denaturation)	95	3 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 chikungunya 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 (Chikungunya)	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 (Chikungunya)	YAKIMA YELLOW/ VIC/ HEX (IC)	Fluorophore / Interpretation	Conclusion
1	√	√	Chikungunya RNA detected	Proceed for further Analysis
2	√	-		
3	-	√	Chikungunya 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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