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

QL-NIV-25 : 25 rxns
QL-NIV-50 : 50 rxns
QL-NIV-100 : 100 rxns

RUO

PI/QLNIV-00

Intended Use:

Quantiplus® Nipah Virus Detection Kit is a Real-Time PCR based in vitro diagnostic assay for detection of Nipah Virus in throat swabs in VTM (Viral transport medium), human plasma, cerebrospinal fluid and urine. The detection is based on the amplification of a specific region within the nucleocapsid protein gene of Nipah Virus.The kit consists of Nipah virus, Internal Control Primers and Probes Mix, Nipah 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:

Nipah virus belongs to the family *Paramyxoviridae*, genus *Henipavirus*. It is a zoonotic virus, spreading between animals and humans. Fruit bats (genus *Pteropus*) is the animal host reservoir for the virus. Infected fruit bats can spread the disease to humans and pigs. People get infected if they have close contact with an infected animal/human or its body fluids. Infections can range from asymptomatic infection to acute respiratory infection (mild, severe), and fatal encephalitis. The case fatality rate is estimated at 40% to 75% based on the outbreaks between 1998-2018. Nipah Virus infection can be very difficult to diagnose owing to the non-specific early symptoms of the illness. However, early detection and diagnosis helps to increase chances of survival among infected individuals, to prevent transmission to other people, and to manage outbreaks.

Kit Components

Color Coding (Caps)	Contents	Description	25 rxns (QL-NIV-25)	50 rxns (QL-NIV-50)	100 rxns (QL-NIV-100)
Green	R Fast Core qPCR Mix (2X)	Amplification Reagent	1 x 325 µL	1 x 650 µL	2 x 650 µL
Amber	Nipah Primer Probe Mix	Nipah, 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	Nipah Positive Control	Nipah Virus 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.

Storage and Transportation Conditions:

The kits 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.

Assay Procedure:

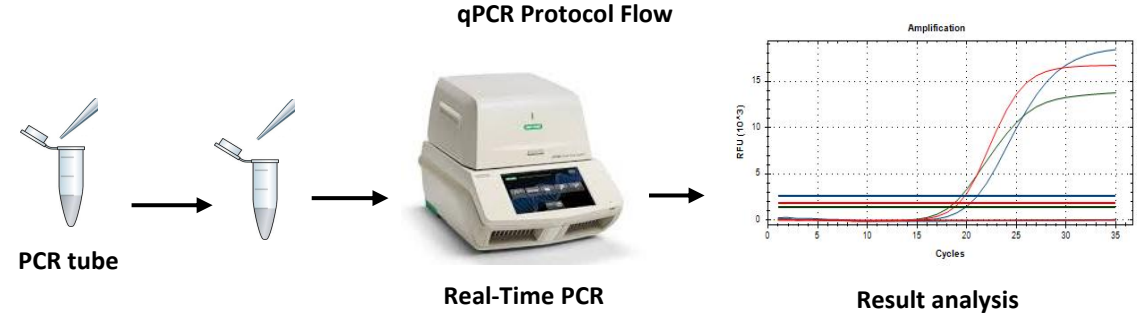
RNA Extraction

Quantiplus® Nipah Virus Detection Kit (Real-Time Qualitative PCR Kit) has been validated using the following Viral RNA extraction kits 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 DNA extraction Kits.

qPCR Protocol Flow



Preparation of Reaction Master mix

Components	Volume per reaction (for 26 µL)
R Fast Core qPCR Mix (2X)	13.0
Nipah Primer-Probe Mix	2.0
Huwel IC-B Mix((if not added at extraction step)	1.0
Extracted RNA/ Nipah 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	53	5 min
2. (Initial denaturation)	1	95	3 min.
3. (PCR cycling)	45	95	10 sec.
		60*	30 sec.
***Plate read/Data acquisition in FAM and TEXAS RED**			

Sample analysis and Interpretation

Interpret the values for unknown samples would appear in the result column in FAM Channel. Samples showing no amplification in FAM channel should show amplification in Texas Red channel, and then only results should be considered. The negative control should not show any value in the FAM channel. It is important to analyze the target channels (FAM and Texas Red) individually in separate windows for better clarity.

S.No	FAM (Nipah)	TEXAS RED(IC)	Fluorophore ← / Interpretation ↓	Conclusion
1	√	√	Nipah RNA detected	Proceed for further Analysis
2	√	-		
3	-	√	Nipah 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 Texas Red) to be analyzed individually.

Validated Instruments

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

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