

Quantiplus® Monkey Pox Virus FAST RT PCR Kit (Real-Time Qualitative PCR Kit)



QLF-MPV-25 : 25 rxns
 QLF-MPV -50 : 50 rxns

PI/QLFMPV-00

Introduction

The Monkeypox Virus real time PCR kit is used for the detection of Monkeypox Virus in serum or lesion exudate samples by using real time PCR systems. This product is a fluorescent probe-based Taqman® real-time PCR assay system. Specific primers and probes are designed for the detection of West African clade, Congo Basin Clade. The kit also detects the presence of general virus from orthopoxvirus genes using specific primer and probes targeting a conserved region of the genus.

Product Description

Quantiplus® Monkey Pox Virus FAST RT PCR Kit is a Real Time PCR based *in-vitro* diagnostic assay for detection of Monkey Pox Virus in human serum or lesion exudate. The kit contains an advanced formulated qPCR mix with UNG/UDG which enables performance of **Fast PCR in shorter run time**, UNG/UDG helps in controlling the PCR carryover contamination, Primer probe mix, positive control, and Exogenous IC.

Kit components

Color Coding (Caps)	Contents	Description	25 rxns (QLF-MPV-25)	50 rxns (QLF-MPV-50)
Green	DNA Fast qPCR Mix with UDG (2X)	PCR Amplification Mix	1 x 325µL	1 x 650µL
Amber	MPV Fast PPM	Target specific Primer probe mix	1 x 50µL	1 x 100µL
Natural	IC-B Mix	Internal Control-B Mix	1 x 300µL	1 x 600µL
Red/Lilac	MPVFPC	Positive Clone	1 x 50 µL	1 x 100 µL
White	MBGPW	Purified water	1 x 500 µL	1 x 500 µL

Storage and Transportation Conditions

The kits could be transported at temperature below -20 °C. The kit will remain stable at least until the expiry date printed on the package, if the storage temperature is kept (-20 ± 5 °C). Kit is stable after 4 repeated freezing/thawing cycles. The reagents should be frozen in aliquots, if they are to be used intermittently.

Technical Specification

Specificity	West African, Congo basin, genes Orthopox Virus
Validated Specimen	Serum, lesion exudates
Target Channel	FAM, HEX, TEXAS RED, Cy5

Assay Procedure

DNA Extraction

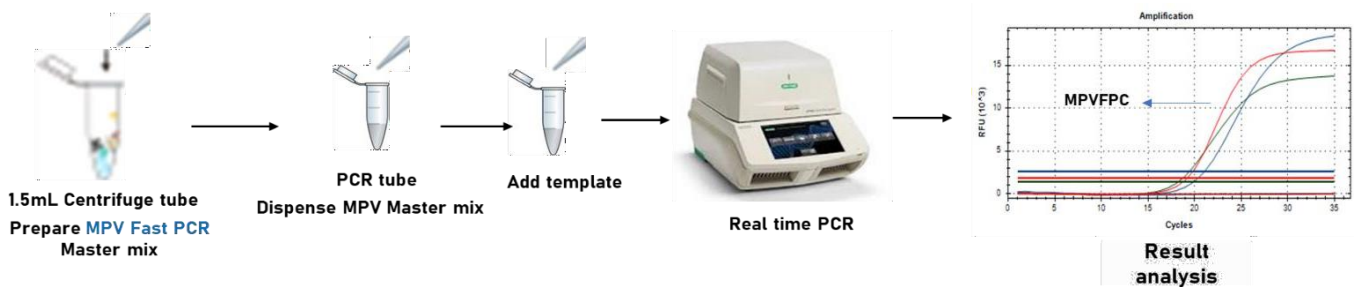
Quantiplus[®] Monkey Pox Virus FAST RT PCR Kit has been validated using the following Viral DNA extraction kits:

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 (Cat. No. HL-NAX-100/250)	200 µL	100 µL
2.	QIAamp DNA Blood Mini Kit (Cat. No. 51104)	200 µL	100 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 26µL)
DNA Fast qPCR Mix with UDG (2X)	13.0
MPV Fast PPM	2.0
IC-B Mix	1.0
Extracted DNA/ MPVFPC / MBGPW	10.0

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

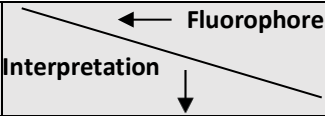
Cycling Conditions

Steps	No. of cycles	Temperature (°C)	Time
1 (Initial denaturation)	1	95	1 min.
2 (PCR cycling)	45	95	10 sec.
		60*	10 sec.

*Plate Read/Data Acquisition in **FAM, HEX, Texas Red and Cy5** Channel

Sample analysis 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.

S.No	FAM (West African)	HEX (Congo Basin)	Texas red (Orthopox genus)	Cy5 (Internal Control)		Conclusion
1	✓	✓	✓	✓	Monkey Pox Virus Positive	Proceed for further Analysis
2	✓	-	✓	✓		
3	-	✓	✓	✓		
4	-	-	✓	✓	Detected positive for genus Orthopox Virus	
5	-	-	-	✓	Monkey Pox Virus Negative	Dilute the DNA sample (1:10) and repeat the Assay
6	-	-	-	-	Invalid Result	

Note : All the Target channels (FAM, HEX, Texas Red and Cy5) to be analyzed individually.

Validated Instruments

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



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Quality management system is certified in compliance with the requirements of the standard ISO 9001:2015