Microzone Mpox Virus Detection Kit

Instructions For Use

MPOX-100 100 Reactions

Manual Date: October 2024

Version Number: 1.0

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Introduction

This kit is for the identification of mpox virus (MPXV) and other orthopoxviruses nucleic acids using Real-Time PCR (qPCR). It targets specific conserved regions of the of the virus's genome. It is intended for research use only by trained laboratory staff.

Kit Contents

Product	Product Code	Quantity
2X MegaMix Platinum Probe qPCR Mastermix	2MMPU-1	1 x 1 mL
Mpox Primer/Probe Mix	PP-MPOX-0.5	1 x 500 µL
Positive Control	PC-MPOX-0.1	1 x 100 µL
Just Water	5JWA-1	1 x 1 mL

Storage and Stability

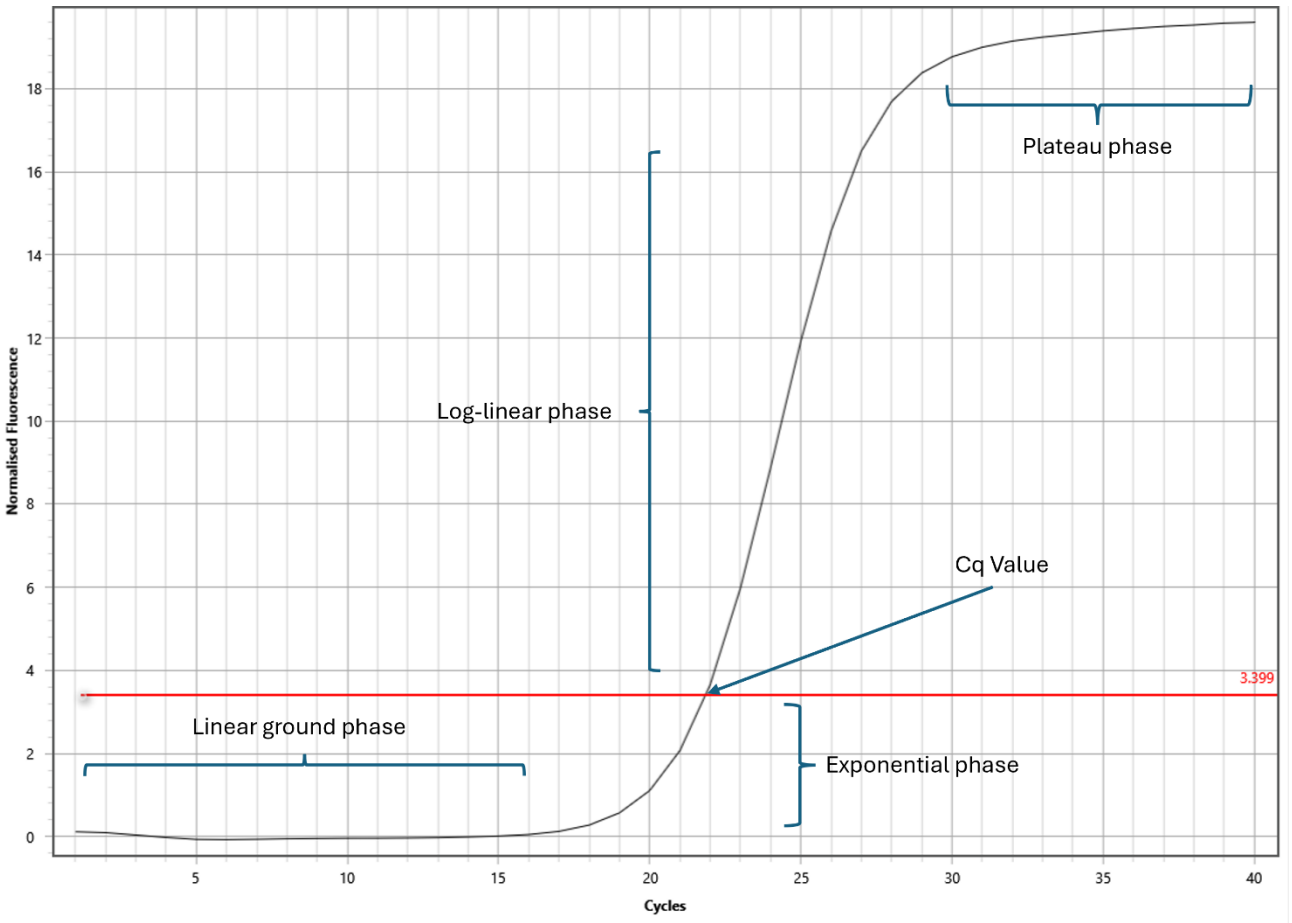
Store the Microzone mpox virus detection kit at -20°C until the expiry date displayed on the label.

Materials Required but Not Provided

- Disposable powder-free nitrile gloves
- Pipettes (adjustable) and sterile filtered pipette tips
- 0.5/1.5 mL nuclease free microcentrifuge tubes
- Benchtop centrifuge
- Vortex mixer
- Decontaminated bench
- Optically clear PCR tubes and caps or 96-well microplate suitable for use with chosen PCR instrument
- Plate sealer (if required)
- qPCR Thermal cycler

Assay Principle

The assay utilises qPCR to detect the presence of specific sequences of DNA found in target of interest. Specifically, the dual-labelled hydrolysis probe technology is employed as a method of detection as it allows the sensitive and specific detection of multiple targets within one reaction. The qPCR amplification consists of 4 phases throughout the run: initially it is a linear ground phase, where there is not sufficient amplified DNA for a change in florescence to be measured; next is an exponential phase, where detectable fluorescence is observed; followed by a Log-linear phase, where the efficiency of the reaction is measured; finally the reaction reaches the plateau phase, where the reaction slows down resulting in a reduced rate of increase in fluorescence as the reaction components deplete.



Linear plot of a qPCR curve.

Technology

Dual-labelled hydrolysis probes are the most widely employed method in quantitative PCR (qPCR) for detecting and quantifying specific DNA sequences. It utilises a fluorescently labelled probe that binds to a complementary target sequence within the PCR product. The probe is a short oligonucleotide, consisting of a reporter dye at the 5’ end and a quencher at the 3’ end. While the probe is intact, the quencher suppresses the fluorescence of the reporter. During the PCR amplification, the 5' to 3' exonuclease activity of Taq polymerase cleaves the probe’s nucleotides, separating the reporter from the quencher, leading to an increase in fluorescence proportional to the amount of product amplified. The increase of fluorescence signal due to the dual-labelled hydrolysis probe technology allows the real-time monitoring of PCR amplification, providing highly specific and sensitive detection of nucleic acids.

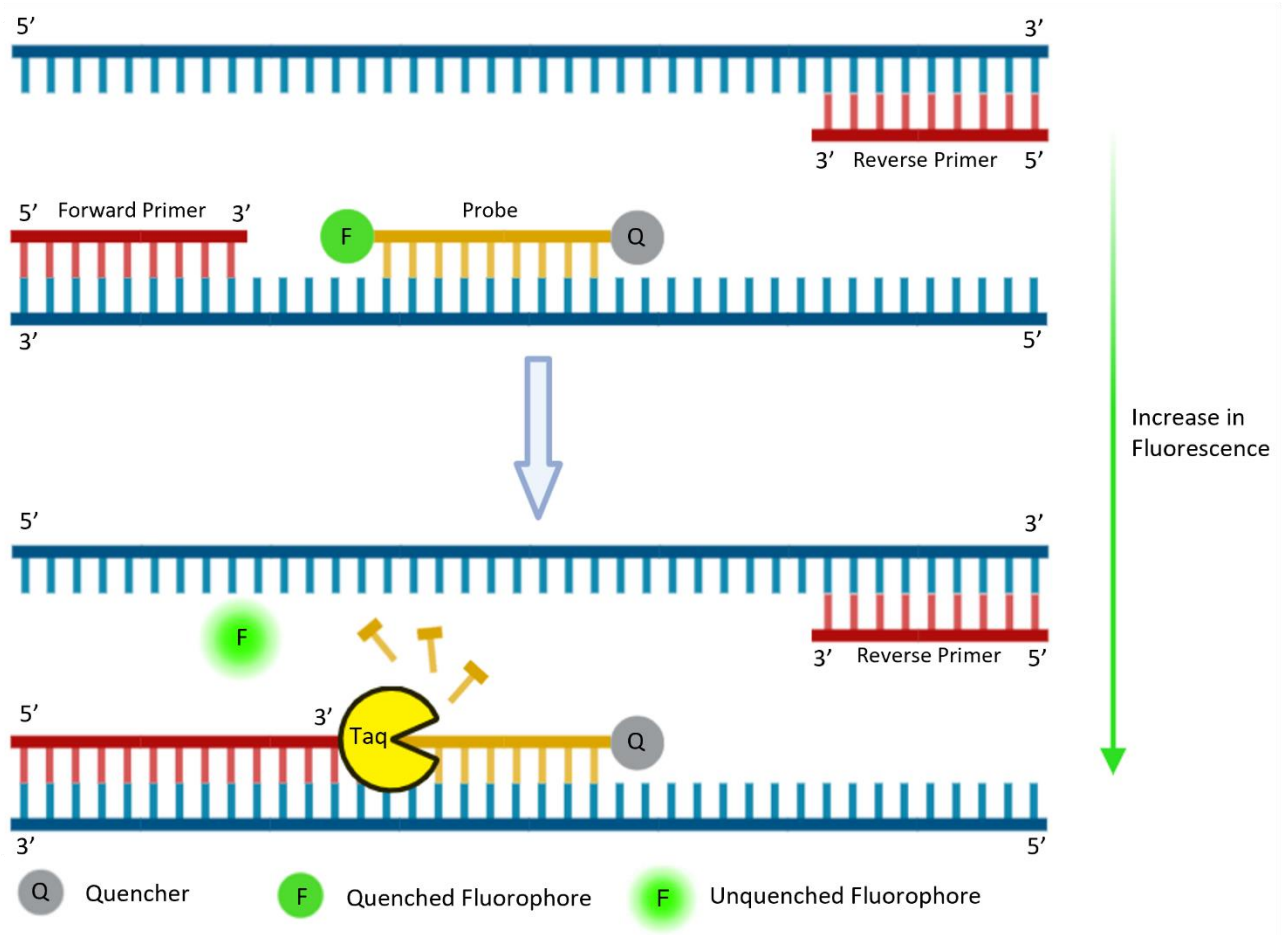


Diagram showing how dual-labelled hydrolysis probes work.

Assay Design

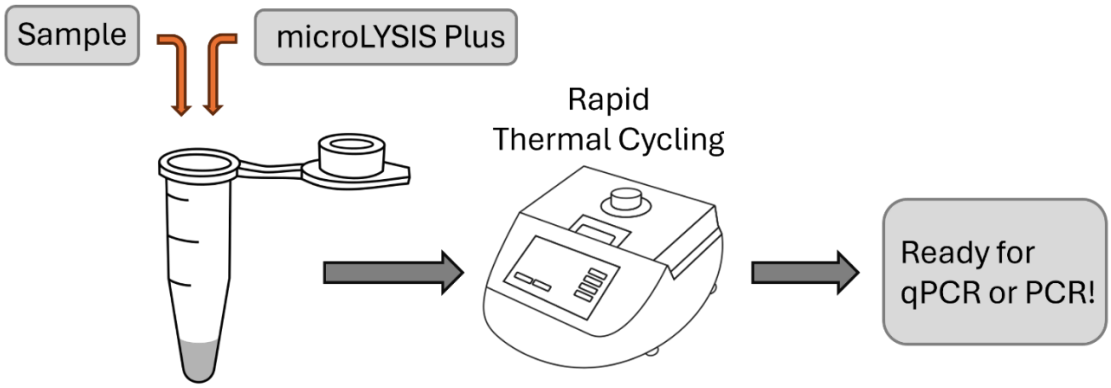
This qPCR assay detects the species-specific F3L gene of the mpox virus, a genus-specific conserved sequence of DNA present in all orthopoxviruses (OPV), and the human RNase P gene which is an internal positive control (IPC). The IPC verifies that the sample has been taken correctly and the conditions in each reaction are compatible with PCR.

Protocol

The protocol consists of three steps: nucleic acid extraction/purification, qPCR and analysis. Nucleic acids are extracted/purified from samples suspected of being mpox virus positive. The purified nucleic acids are then amplified using MegaMix Platinum qPCR Mastermix and mpox Primer/Probe Mix. Analysis of the qPCR amplification allows the user to determine if the tested sample contained mpox virus or other orthopoxviruses nucleic acids.

Nucleic Acid Extraction/Purification

This kit can be used with tradition nucleic acid extraction methods that utilise magnetic beads or columns and alternatively, it can be used with a direct-to-PCR lysis buffer such as microLYSIS Plus from Microzone. microLYSIS Plus significantly shortens the sample preparation process, with only a short heat step to release and stabilise nucleic acids ready for PCR. More information about microLYSIS Plus can be found here: https://microzone.co.uk/shop/extraction/microlysis-plus/.



Reaction Setup

1. Thaw all reagents at room temperature.
2. Once thawed vortex all reagents and briefly centrifuge to remove any liquid from the cap.
3. Every reaction set up must allocate sufficient wells for the number of samples, a positive, and a negative control (samples + 2 = total wells needed). For example, 10 samples to test + 1 Positive control well + 1 negative control well = 12 reaction wells required.
4. Setup should be done on ice when possible. New sterile filter pipette tips are to be used between each pipetting.
5. Generate the following mastermix, use the number of reactions well determined in step 1, adding 5% excess (to allow for pipetting loss) to calculate volumes:

Component	Volume
MegaMix Platinum qPCR Mastermix with UNG	10 µL
Primer and probe mix	5 µL
Total volume of mastermix per sample	**15 µL**

6. Briefly vortex to mix.

7. Dispense 15 µL of mastermix into each reaction well.
8. Add 5 µL of sample to each well containing mastermix; for the positive control and negative control add 5 µL Positive Control and 5 µL Just Water, respectively.
9. Cap the tube with corresponding cap.
10. Briefly centrifuge to move all of the reaction to the base of the tube (some instruments this is not required as it is done during the PCR).
11. Transfer the tubes to the PCR instrument.

Thermal cycling

Run the following thermal cycling profile:

Temperature	Time	Cycles	Fluorescence collection	Step
95°C	2 minutes	1		Initial Denaturation
95°C	3 seconds	42		Denaturation
60°C	20 seconds		FAM, CY5, HEX	Annealing/Extension

Interpretation of Results

Target	Reporter Fluorophore	Sample	
		Cq Value	Interpretation
F3L	FAM	<40	Positive
		≥40 or No amplification	Negative
OPV	Cy5	<40	Positive
		≥40 or no amplification	Negative
RNase P (IPC)	HEX	<30	Positive
		≥30 or no amplification	Negative

	FAM	Cy5	HEX (IPC)	Reporting
Positive Control	+	+	+	Valid
Negative Control	-	-	-	Valid
Case 1	+	+	+/-	Mpox positive
Case 2	-	+	+/-	Orthopoxvirus positive and mpox virus negative
Case 3	-	-	+	Orthopoxvirus and mpox virus negative
Case 4	+	-	+/-	Inconclusive
Case 5	-	-	-	Invalid

Note: Any other combination of Positive or Negative Control result invalidates the run.