

Primerdesign

R01340

Mpox

Differentiation of clades Ia, Ib and II

Kit version: 1

Target regions

Mpox all clades: G2R gene

Mpox clade Ia: OPG032/C3L gene

Mpox clade Ib: OPG151/rpo132 gene

Mpox clade II: G2R gene

genesig[®] PLEX Kit

100 tests

GENESIG

Kits by Primerdesign

For general laboratory and research use only

Product Description

The genesig®PLEX qPCR detection kit detects and differentiates clades Ia, Ib and II of mpox virus. Infection with mpox virus can lead to fever, headache, swollen lymph nodes, fatigue and muscle aches along with characteristic pox lesions. The fatality rate of human mpox virus can range from 1% up to 11%, with younger age groups having the highest rate.

Specificity

The kit is designed for the in vitro detection of mpox virus clades Ia, Ib and II genomes with a broad detection profile for these pathogens. Specifically, the primers will detect over 95% of sequences available on the NCBI database at the time of last review.








The mpox virus all clades assay is predicted to cross react with Akhmeta virus and Orthopoxvirus Abatino. This would produce a fluorescence signal in the ROX channel.

The mpox virus clade Ia assay is predicted to cross react with Cowpox virus, Camelpox virus, Vaccinia virus, Variola virus, Ectromelia virus and Taterapox virus. This would produce a fluorescence signal in the Cy5 channel.

The mpox virus clade Ib assay is predicted to cross react with Cowpox virus, Camelpox virus, Vaccinia virus (including Buffalopox and Horsepox viruses), Variola virus, Taterapox virus, Ectromelia virus, Akhmeta virus and Orthopoxvirus Abatino. This would produce a fluorescence signal in the FAM channel.

The dynamics of genetic variation means that new sequence information may become available after the initial design. If you require further information or have a specific question about the detection profile of this kit then please send an e-mail to techsupport@primerdesign.co.uk and our team will answer your question.

Kit contents

Quantity	Component	Tube	Cap Colour
1	Mpox (clade Ia, Ib, II) primer/probe mix (including IEC primer/probe mix) (100 reactions) Mpox all clades (ROX) Mpox clade Ia (Cy5) Mpox clade Ib (FAM) Mpox clade II (Cy5.5) IEC (VIC)		BROWN
1	Mpox (clade Ia, Ib, II) positive control template		RED (in silver foil wrapper)
2	oasig® Lyophilised 2X qPCR Master Mix (50 reactions per glass vial)		SILVER
2	oasig® Master Mix resuspension buffer		BLUE
1	genesig® Easy DNA internal extraction control		BLUE (in gold foil wrapper)
1	Template preparation buffer for resuspension of internal control template and positive control template		YELLOW
1	RNase/DNase free water for resuspension of primer/probe mixes		WHITE

Reagents and equipment to be supplied by the user

Real-time PCR Instrument

Must be able to read fluorescence through FAM, Cy5, ROX, Cy5.5 and HEX/VIC.

Extraction kit

This kit is recommended for use with genesig® Easy DNA/RNA extraction kit or exsig®Mag. However, it is designed to work well with all processes that yield high-quality nucleic acid with minimal PCR inhibitors.

Pipettors and filter tips

Vortex and centrifuge

1.5 ml microtubes

qPCR plates or reaction tubes

Kit storage and stability

This kit is stable for shipping at ambient temperature but should be stored at -20°C upon arrival. Once the lyophilised components have been resuspended, they should not be exposed to temperatures above -20°C for longer than 30 minutes at a time and unnecessary repeated freeze/thawing should be avoided. The kit is stable for six months from the date of resuspension under these circumstances.

Primer Design Ltd does not recommend using the kit after the expiry date stated on the pack.

Suitable sample material

This kit can be used with all types of samples from various origins. Please ensure that the extracted nucleic acid samples are suitable in terms of purity, concentration, and DNA/RNA integrity. An internal extraction control is provided to test for non-specific PCR inhibitors.

Dynamic range of test

Under optimal PCR conditions the kit can achieve priming efficiencies between 90-110% and detect less than 100 copies of target template.

Principles of the test

Real-time PCR

Individual primers and probes designed for each pathogen have been combined into one reaction and these can be detected through the different fluorescent channels as described in the kit contents.

The assay consists of labelled probes in one test specific for mpox virus all clades in ROX, mpox virus clade Ia in Cy5, mpox virus clade Ib in FAM and mpox virus clade II in Cy5.5.

The assay includes an internal extraction control (genesig® Easy DNA internal extraction control), which may be added to the nucleic acid extraction system (not provided) to prove efficient DNA extraction, detect PCR inhibition and confirm the integrity of the PCR run. The internal extraction control assay (which is from a non-biologically relevant exogenous source) is present in both tests and the probe is labelled with the VIC fluorophore.

The primer and probe mix provided exploits the so-called TaqMan® principle. During PCR amplification, forward and reverse primers hybridise to the target cDNA. A fluorogenic probe is included in the same reaction mixture which consists of a DNA probe labelled with a 5'-dye and a 3'-quencher. During PCR amplification, the probe is cleaved, and the reporter dye and quencher are separated. The resulting increase in fluorescence can be detected on a range of qPCR platforms.

Positive control

The kit contains one positive control that contains target templates. The positive control contains templates for mpox virus all clades, mpox virus clade Ia, mpox virus clade Ib and mpox virus clade II. Each time the kit is used, at least one positive control reaction must be included in the run. A positive result indicates that the primers and probes for detecting the target gene worked properly in that particular experimental scenario. If a negative result is obtained, the test results are invalid and must be repeated. Care should be taken to ensure that the positive control does not contaminate any other kit component which would lead to false-positive results. This can be achieved by handling this component in a post PCR environment. Care should also be taken to avoid cross-contamination of other samples when adding the positive control to the run. This can be avoided by sealing all other samples and negative controls before pipetting the positive control into the positive control well.

Negative control

To validate any positive findings a negative control reaction should be included every time the kit is used. For this reaction the RNase/DNase free water should be used instead of template. A negative result indicates that the reagents have not become contaminated while setting up the run. It is also known as a No Template Control or NTC.

Internal DNA extraction control

When performing DNA extraction, it is often advantageous to have an exogenous source of DNA template that is spiked into the lysis buffer. This control DNA is then co-purified with the sample DNA and can be detected as a positive control for the extraction process. Successful co-purification and qPCR for the control DNA also indicates that PCR inhibitors are not present at a high concentration.

The Mpox (clade Ia, Ib, II) primer/probe mixes contain a specific primer and probe mix to detect the exogenous DNA using qPCR. The primers are present at PCR limiting concentrations which allows multiplexing with the target sequence primers. Amplification of the control DNA does not interfere with detection of the target DNA even when present at low copy number. The Internal control is detected through the VIC channel and gives a Cq value of 28 \pm 3 depending on the level of sample dilution.

Resuspension protocol

To minimise the risk of contamination with foreign DNA, we recommend that all pipetting is performed in a PCR clean environment. Ideally this would be a designated PCR lab or PCR cabinet. Filter tips are recommended for all pipetting steps.

1. Pulse-spin each tube in a centrifuge before opening.

This will ensure lyophilised primer/probe mix or template is in the base of the tube and is not lost upon opening the tube.

2. Resuspend the primer/probe mix in the RNase/DNase free water supplied, according to the table below:

To ensure complete resuspension allow primer/probe mix to rehydrate for 10 minutes at room temperature. Vortex each tube thoroughly, followed by pipetting up and down 10 times. Failure to mix well can produce poor kit performance.

Component - resuspend in water	Volume
Pre-PCR pack	
Mpox (clade Ia, Ib, II) primer/probe mix (BROWN)	110 µl

3. Resuspend the internal control template and positive control template in the template preparation buffer supplied, according to the table below:

To ensure complete resuspension, vortex each tube thoroughly.

Component - resuspend in template preparation buffer	Volume
Pre-PCR heat-sealed foil	
Internal extraction control DNA (BLUE)	600 µl
Post-PCR heat-sealed foil	
Mpox (clade Ia, Ib, II) positive control template (RED) *	500 µl

* This component contains high copy number template and is a VERY significant contamination risk. It must be opened and handled in a separate laboratory environment, away from the other components.

4. Resuspend the oasis[®] Lyophilised 2X qPCR Master Mix in oasis[®] resuspension buffer, according to the table below:

Component - resuspend in template preparation buffer	Volume
oasis [®] Lyophilised 2X qPCR Master Mix (SILVER)	525 µl

Nucleic acid extraction

The internal extraction control DNA can be added either to the nucleic acid lysis/extraction buffer or to the sample once it has been resuspended in lysis buffer.

DO NOT add the internal extraction control DNA directly to the unprocessed biological sample as this will lead to degradation and a loss in signal.

1. Add 4 µl of the Internal extraction control DNA (**BLUE**) to each sample in DNA lysis/extraction buffer.
2. Complete nucleic acid extraction according to the manufacturer's protocols.

RT-qPCR detection protocol

For optimum performance and sensitivity.

All pipetting steps and experimental plate set up should be performed on ice. After the plate is prepared proceed immediately to the amplification protocol. Prolonged incubation of reaction mixes at room temperature can lead to PCR artifacts that reduce the sensitivity of detection.

1. For each DNA sample prepare a reaction mix according to the table below:
Include sufficient reactions for all samples, positive and negative controls.

Component	Volume
oasig [®] Lyophilised 2X qPCR Master Mix (SILVER)	10 µl
Mpox (clades Ia, Ib and II) primer/probe mix (BROWN)	1 µl
RNase/DNase free water (WHITE)	4µl
Final Volume	15 µl

2. Pipette 15 µl of these mixes into each well according to your experimental qPCR plate set-up.
3. Pipette 5 µl of extracted sample into each well according to your experimental plate set-up.

For negative control wells use 5 µl of RNase/DNase free water (**WHITE**). For positive control wells use 5 µl of the positive control template (**RED**). The final volume in each well is 20 µl.

qPCR amplification protocol

Amplification conditions for oasisig[®] Lyophilised 2X qPCR Master Mix (**SILVER**)

	Step	Time	Temp
	Enzyme activation	2 min	95 °C
Cycling x50	Denaturation	10 s	95 °C
	DATA COLLECTION *	60 s	60 °C

* Fluorogenic data should be collected during this step through the ROX, Cy5, FAM, Cy5.5 and VIC channels.

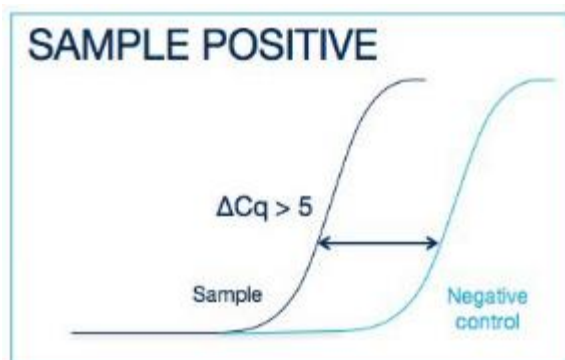
Interpretation of results

Mpox (clade Ia, Ib and II) reaction mix:

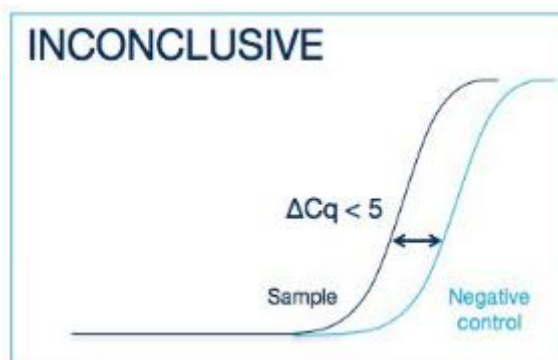
Target (FAM/Cy5/Cy5.5)	Mpox virus all clades (ROX)	Internal control (VIC)	Positive control	Negative control	Interpretation
Cy5+	+	+ / -	+	-	Mpox virus clade Ia POSITIVE RESULT
FAM+	+	+ / -	+	-	Mpox virus clade Ib POSITIVE RESULT
Cy5.5+	+	+ / -	+	-	Mpox virus clade II POSITIVE RESULT
+	-	+	+	-	NEGATIVE RESULT *
-	-	+	+	-	NEGATIVE RESULT
+ / -	+ / -	+ / -	+	≤ 35	EXPERIMENT FAILED due to test contamination
+ / -	+ / -	+ / -	+	> 35	**
-	-	-	+	-	SAMPLE PREPARATION FAILED
+ / -	+ / -	+ / -	-	+ / -	EXPERIMENT FAILED

* Where there is a positive result for an mpox clade but negative for the mpox all clades assay, the result must be considered as negative as this is potentially due to cross reactivity with a non-mpox orthopoxvirus.

** Where the test sample is positive and the negative control is positive with a Cq > 35, the sample must be reinterpreted based on the relative signal strength of the two results:



If the sample amplifies > 5 Cq earlier than the negative control, then the sample should be reinterpreted (via the table above) with the negative control verified as negative.



If the sample amplifies < 5 Cq earlier than the negative control, then the positive sample result is invalidated, and the result should be determined inconclusive due to test contamination. The test for this sample should be repeated.

Positive Control

The positive control tube contains all the templates for the targets detected by that tube and should produce positive amplification plots in the ROX, Cy5, FAM and Cy5.5 channels. There is no internal control template within the positive control so the VIC channel should give no signal (flat amplification plot). The positive control signals indicate that the kit is working correctly to detect each target.

The positive control is expected to amplify between Cq 16 – 23 in the ROX, Cy5, FAM and Cy5.5 channels. Failure to satisfy this quality control criterion is a strong indication that the experiment has been compromised and should be repeated.

No template control (NTC)

The NTC should give a flat line (flat amplification plots) through all channels. Signals in the NTC indicate cross contamination during plate set up.

DNA Internal extraction control

The Cq value obtained with the internal control will vary significantly depending on the extraction efficiency, the quantity of nucleic acid in the PCR reaction and the individual machine settings. Cq values of 28 ± 3 are within the normal range. When amplifying a target sample with a high genome copy number, the internal extraction control may not produce an amplification plot. This does not invalidate the test and should be interpreted as a positive experimental result.

Sample data

Presence of the target pathogens is shown by amplification in the channels indicated in the kit contents section. Positive signals indicate positive tests for those targets. It may be possible for samples to contain multiple targets, therefore positive results in the ROX, Cy5, FAM and Cy5.5 channels may be present.

Notices and disclaimers

This product is developed, designed and sold for research purposes only. It is not intended for human diagnostic or drug purposes or to be administered to humans unless clearly expressed for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. During the warranty period, Primer Design Ltd genesig® detection kits allow precise and reproducible data recovery combined with excellent sensitivity. For data obtained in violation of the general GLP guidelines and the manufacturer's recommendations, the right to claim under guarantee is expired.

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