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. Mpox virus (formerly called monkeypox virus), also known as MOXV, is a zoonotic DNA virus in the Orthopoxvirus genus, related to smallpox. There are two distinct clades of the virus: clade I (with subclades Ia and Ib) and clade II (with subclades IIa and IIb). The mpox disease typically spreads through close contact with infected animals or people, including direct contact with lesions, bodily fluids, or contaminated materials. Symptoms often include fever, swollen lymph nodes, fatigue, and a characteristic rash. While most cases are mild and self-limiting, severe illness can occur, particularly in immunocompromised individuals and children, and can be fatal. Clade I has a higher fatality 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 GISAID EpiPox database at the time of last review.

Due to sequence homology of the mpox virus with other Orthopoxviruses, some cross-reactivity is predicted as below:

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 the primer/probe mix		**WHITE**

Reagents and equipment to be supplied by the user

Real-time PCR Instrument

Must be able to read fluorescence through FAM, ROX, Cy5, 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

This kit is designed for the detection of the target using real-time PCR (also known as qPCR). Probe-based real-time PCR is a sensitive and specific detection method, where the target DNA is amplified and simultaneously measured during each PCR cycle. Target-specific primers hybridise to the target DNA and initiate amplification. A fluorogenic probe is included with the primer/probe mix, which consists of a target-specific oligo sequence labelled with a 5'-dye and 3'-quencher. When the target is present, the probe is cleaved during PCR amplification, and the reporter dye and quencher are separated, resulting in an increase in fluorescence that can be detected by the real-time PCR instrument.

This genesig®PLEX assay consists of multiple primers and labelled probes, specific for mpox virus all clades (ROX), mpox virus clade Ia (Cy5), mpox virus clade Ib (FAM) and mpox virus clade II (Cy5.5). The individual primers and probes designed for each of the clades have been combined into one reaction and can be detected through the different fluorescent channels.

Positive control

It is recommended to include a positive control in every run. For this, a positive control template is provided in the kit, which contains the target region of each assay (Mpox all clades, clade Ia, clade Ib, and clade II). A positive result indicates that the reagents and PCR run worked as expected. The positive control template should be handled with care to avoid contamination of other components, samples, or the environment, which could lead to false-positive results. This can be achieved by handling the positive control template in a designated positive control area, and by sealing samples and negative controls before preparation of the positive control.

Negative control

It is recommended to include a negative control, also known as a No Template Control (NTC), in every run. For this, the RNase/DNase free water included in the kit should be used instead of a sample. A negative result indicates that the reagents have not become contaminated while setting up the run and thereby verifies any positive sample results.

Internal DNA extraction control

When extracting nucleic acids from a sample, it is advantageous to include an exogenous control that is spiked into the sample during the lysis stage, as a control for the full workflow. For this, an internal extraction control DNA template is included in the kit. The template reflects the nature of the target (DNA) and is co-extracted with the sample. The Mpox (clade Ia, Ib, II) primer/probe mix contains a specific primer and probe mix to detect the internal extraction control template through the VIC channel in the same reaction as the mpox targets during qPCR. The primers are present at PCR limiting concentrations, which allows multiplexing with the target assays without interference or competition. Amplification of the internal extraction control indicates the efficiency of extraction, quality and purity of the extract, and absence of PCR inhibitory effects, which is important to verify negative samples.

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 oasig® Lyophilised 2X qPCR Master Mix in oasig® resuspension buffer, according to the table below:**

Component - resuspend in oasig® resuspension buffer	Volume
oasig® 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.**

qPCR detection protocol

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 this mix 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 oasig® Lyophilised 2X qPCR Master Mix (SILVER)

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

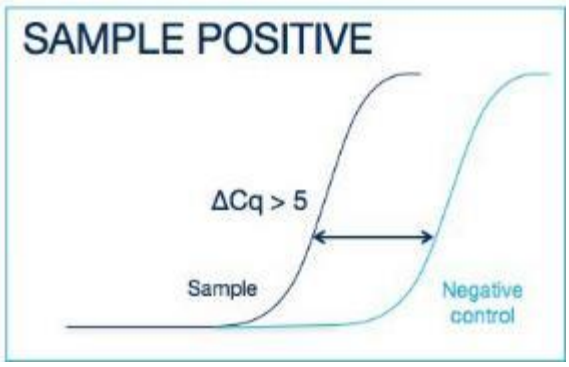
* Fluorogenic data should be collected during this step through the ROX, Cy5, FAM, Cy5.5 and VIC channels.

Interpretation of results

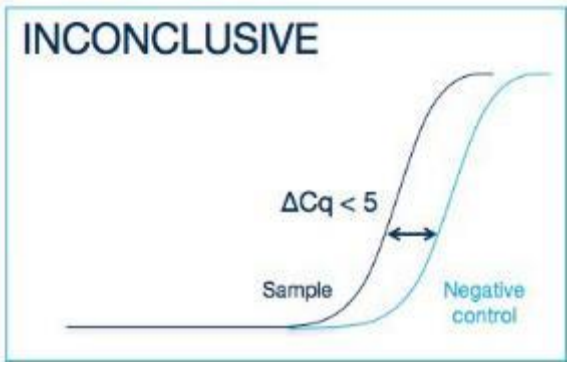
Target (FAM/Cy5/Cy5.5)	Mpox virus all clades (ROX)	Internal control (VIC)	Positive control	Negative control	Interpretation
Cy5+	**+**	**+ / -**	**+**	**-**	**Mpox virus clade Ia POSITIVE RESULT**
FAM+	**+**	**+ / -**	**+**	**-**	**Mpox virus clade Ib POSITIVE RESULT**
Cy5.5+	**+**	**+ / -**	**+**	**-**	**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 the target region for all mpox targets detected by the kit 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.

Negative Control

The negative control is expected to be free of amplification. If the negative control shows amplification, please refer to the interpretation table for guidance, as this indicates contamination.

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 Cy5, FAM and Cy5.5 channels may be present, with concurrent amplification of mpox all clades in ROX.

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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