Primerdesign

R00948

Mpox 2G

Kit version: 1

Target region:

Mpox virus (OPG168/OPG169)

Orthopoxvirus (rpo132)

genesig® Advanced DNA Kit

150 tests

GENESIG

Kits by Primerdesign

For general laboratory and research use only

Product Description

This genesig® Advanced qPCR detection kit targets the OPG168/OPG169 region from mpox which was previously known as MPXV or Monkeypox. Mpox is a DNA virus in the Orthopoxvirus genus, which is thought to primarily infect rodents, but can infect humans, and may also infect non-human-primates and other mammals. Infection can lead to mucosal lesions, swollen lymph nodes, and fever, among other symptoms. The species is divided into two clades (I and II), further broken down to Ia, Ib, IIa and IIb, of which all are detected by the qPCR kit. Clade Ib arose from recent mutations and, as of 14th August 2024, has been declared an international health emergency by the World Health Organisation.

Specificity

The kit is designed for the in vitro detection of mpox with a broad detection profile. Specifically, the primers will detect over 95% of sequences available on the GISAID EpiPox database at the time of last review.

The mpox virus primers and probe may show cross reactivity with cowpox virus and vaccina virus (seen as amplification in FAM) but this would produce significantly later Cqs than the Orthopoxvirus assay in Cy5. Please see interpretation of results for more information.

The Orthopoxvirus primer and probe set is designed to detect mpox virus, cowpox virus and vaccina virus genomes, but may cross-react with other members of the Orthopoxvirus genus.

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 2G primer/probe mix (150 reactions)** FAM labelled for Mpox; Cy5 labelled for Orthopoxvirus.		BROWN
1	**Mpox 2G positive control template**		RED
1	**Internal extraction control primer/probe mix (150 reactions)** VIC labelled as standard		BROWN
1	**Internal extraction control DNA (150 reactions)**		BLUE
1	**Endogenous control primer/probe mix (150 reactions)** FAM labelled, Target: Human ACTB as standard		BROWN
1	**RNase/DNase free water** for resuspension of primer/probe mixes		WHITE
3	**Template preparation buffer** for resuspension of internal control template, positive control template and standard curve preparation		YELLOW

Reagents and equipment to be supplied by the user

Real-time PCR Instrument

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.

oasig® lyophilised or PrecisionPLUS® 2X qPCR Master Mix

This kit is intended for use with oasig® lyophilised or PrecisionPLUS® 2X qPCR Master Mix.

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 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

The assay consists of primers and labelled probes multiplexed in a single tube, for mpox amplification in the FAM channel and Orthopoxvirus amplification in the Cy5 channel.

The primer and probe mix provided exploits the so-called TaqMan® principle. During PCR amplification, forward and reverse primers hybridize to the target DNA. 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 a positive control template, which will give amplification in both the FAM and Cy5 channels. 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.

A separate primer and probe mix is supplied with this kit 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+/-3 depending on the level of sample dilution.

Endogenous control

A primer/probe mix for detection of the endogenous control gene is included in the kit, which allows confirmation of a valid biological sample from the host. Detection of the endogenous control is through the FAM channel, and it is therefore NOT possible to perform a multiplex reaction with the target specific primer/probe mix. Amplification of the endogenous control may depend on the sample type used. Please note that if samples from a different species are used, the endogenous control may not be appropriate, but the internal extraction control is advised to be used.

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 mixes 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 2G primer/probe mix (**BROWN**)	165 µl
Internal extraction control primer/probe mix (**BROWN**)	165 µl
Endogenous control primer/probe mix (**BROWN**)	165 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 2G 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.

DNA extraction

The internal extraction control DNA can be added either to the DNA lysis/extraction buffer or to the DNA 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 DNA 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 positive and negative controls.

Component	Volume
oasig® lyophilised or PrecisionPLUS® 2X qPCR Master Mix	10 µl
Mpox 2G primer/probe mix (**BROWN**)	1 µl
Internal extraction control primer/probe mix (**BROWN**)	1 µl
RNase/DNase free water (WHITE)	3 µl
Final Volume	**15 µl**

2. **(Optional) For each DNA sample prepare an endogenous control reaction according to the table below.**
   This control reaction will provide useful information regarding the quality of the biological sample.

Component	Volume
oasig® lyophilised or PrecisionPLUS® 2X qPCR Master Mix	10 µl
Endogenous control primer/probe mix (**BROWN**)	1 µl
RNase/DNase free water (WHITE)	4 µl
Final Volume	**15 µl**

3. **Pipette 15 µl of these mixes into each well according to your experimental plate set up.**

4. **Pipette 5 µl of DNA template 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

Recommended amplification conditions when using oasig® lyophilised or PrecisionPLUS® 2X qPCR Master Mix.

	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 FAM, Cy5 and VIC channels

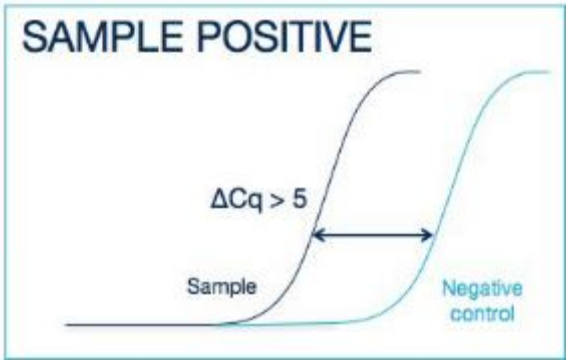
Interpretation of results

Mpox virus (FAM)	Orthopoxvirus (Cy5)	Internal control (VIC)	Positive control	Negative control	Interpretation
+	+	+ / -	+	-	**POSITIVE RESULT FOR MPOX VIRUS ***
-	+	+ / -	+	-	**POSITIVE RESULT FOR ORTHOPOXVIRUS, NEGATIVE FOR MPOX VIRUS**
+	-	+ / -	+	-	**INCONCLUSIVE RESULT**
-	-	+	+	-	**NEGATIVE RESULT**
+ / -	+ / -	+ / -	+	≤ 35	**EXPERIMENT FAILED** due to test contamination
+ / -	+ / -	+ / -	+	> 35	**
-	-	-	+	-	**SAMPLE PREPARATION FAILED**
+ / -	+ / -	+ / -	-	+ / -	**EXPERIMENT FAILED**

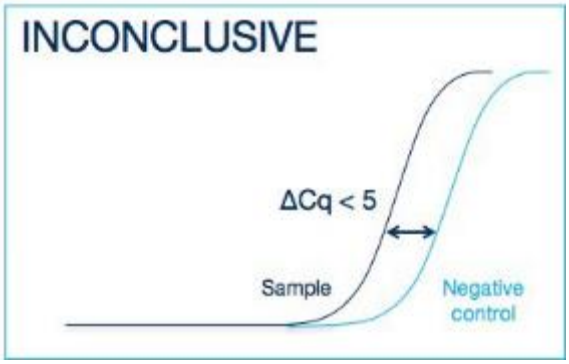
* If sample is positive for both mpox (FAM) and orthopox (Cy5), a sample must be interpreted as follows:

- If the amplification in FAM (mpox) occurs > 5 Cq later than in Cy5 (orthopox), the sample should be considered positive for orthopox and negative for mpox.
- If the amplification in FAM (mpox) occurs within 5 Cq of Cy5 (orthopox), the sample should be considered positive for mpox.

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

Internal PCR control

The Cq value obtained with the internal control will vary significantly depending on the extraction efficiency, the quantity of DNA added to 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.

Endogenous control

The signal obtained from the endogenous control primer and probe set will vary according to the amount of biological material present in a given sample. An early signal indicates the presence of a good yield of biological material. A late signal suggests that little biological material is present in the sample.

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 by violation to the general GLP guidelines and the manufacturer's recommendations the right to claim under guarantee is expired.

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