Primerdesign

R00948

Mpox

Kit version: 1

Target region:

Mpox virus (OPG168/OPG169)

Orthopoxvirus (rpo132)

genesig® Advanced Kit

150 tests

GENESIG

Kits by Primerdesign

For general laboratory and research use only

Introduction to mpox

The genus orthopoxvirus, in the Poxviridae family, includes mpox virus, cowpox virus, camelpox virus, mousepox virus, variola virus (causative agent of smallpox) and vaccinia virus (used for the smallpox vaccine). Transmission primarily occurs by zoonotic transfer from other animals to humans, but human-to-human transmission can occur from contact with skin lesions, body fluids and respiratory secretions of infected individuals. Contact with contaminated clothing and bedding of those infected can also lead to transmission of the virus. The fatality rate of human mpox can range from 1% up to 11%, with younger age groups having the highest rate.

.

There are two clades of mpox virus, the Congo Basin clade (associated with higher human-to-human transmission and higher mortality) and the West African clade (associated with milder infection). Most cases are reported from the Democratic Republic of the Congo and Nigeria. Since May 2022 cases have been reported in Europe, North America, and other parts of the globe.

Clinical presentations in humans are similar to smallpox with fever, headache, swollen lymph nodes, fatigue and muscle aches along with characteristic pox lesions. The incubation period from infection to the onset of symptoms can be between 5-21 days with 6-13 days being the average. PCR tests of skin lesions (surface and/or exudate, roofs or crusts) is the preferred diagnostic testing method for virus detection. The World Health Organization (WHO) also encourage the additional collection of oropharyngeal swabs from the patient, although the accuracy of such samples in detecting the presence of mpox virus is limited. Antigen detection methods are not recommended due to serological cross-reactivity and do not provide mpox virus-specific detection.

Specificity

The genesig® Advanced Kit for mpox is designed for the in vitro detection and quantification of mpox virus and orthopoxvirus genomes. The kit is designed to have a broad detection profile. Specifically, the primers will detect over 95% of sequences available on the NCBI database at the time of last review.

The mpox virus primer and probe set is designed to detect exclusively mpox virus genomes.

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

The dynamics of genetic variation mean that new sequence information may become available after the most recent review. 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, Target: Mpox virus Cy5 labelled, Target: 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 gene 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 and positive control template		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 DNA 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 at room temperature but should be stored at -20ºC on 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

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

The primer and probe mix provided exploits the so-called TaqMan® principle. During PCR amplification, forward and reverse primers hybridise to the target DNA. Fluorogenic probes are 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

For a positive control, the kit contains a single positive control that contains templates for the 2 targets in the test. The kit positive control will give a mpox virus signal through the FAM channel and an orthopoxvirus signal through the Cy5 channel. 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 each target are working properly in that particular run. 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 these components 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 minimize 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 the 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, vortex each tube thoroughly.

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 template (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 4µl of the Internal extraction control DNA (BLUE) to each sample in DNA lysis/extraction buffer per sample.**

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

4. **Pipette 5µl of DNA template into each well, according to your experimental plate set up.**
   For negative control wells use 5µl of RNase/DNase free water (**WHITE**). For positive control wells use 5µl of the positive control template (**RED**). The final volume in each well is 20µl.

qPCR Amplification Protocol

Amplification conditions using oasig® lyophilised or PrecisionPLUS® 2X qPCR Master Mix.

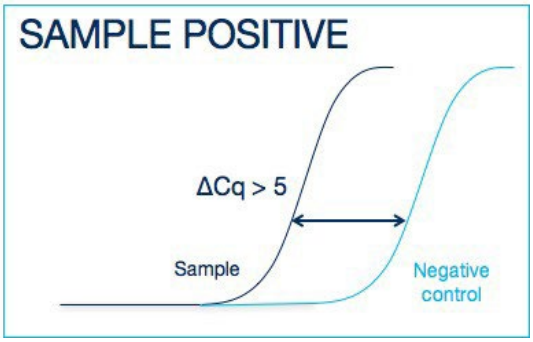
	Step	Time	Temp
	Enzyme activation	2 min	95 °C
Cycling x50	Denaturation	10 s	95 °C
	DATA COLLECTION *	60 s	60 °C

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

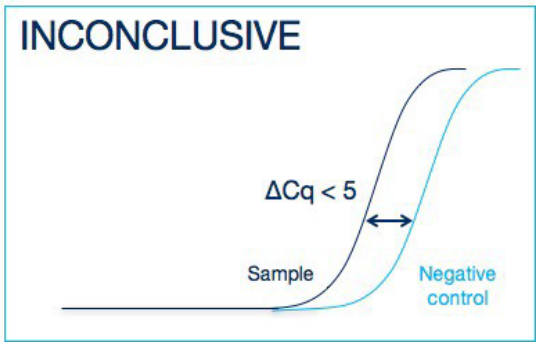
Interpretation of results

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**

* 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 both 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. PCR is a proprietary technology covered by several US and foreign patents. These patents are owned by Roche Molecular Systems Inc. and have been sub-licensed by PE Corporation in certain fields. Depending on your specific application you may need a license from Roche or PE to practice PCR. Additional information on purchasing licenses to practice the PCR process may be obtained by contacting the Director of Licensing at Roche Molecular Systems, 1145 Atlantic Avenue, Alameda, CA 94501 or Applied Biosystems business group of the Applera Corporation, 850 Lincoln Centre Drive, Foster City, CA 94404. In addition, the 5' nuclease assay and other homogeneous amplification methods used in connection with the PCR process may be covered by U.S. Patents 5,210,015 and 5,487,972, owned by Roche Molecular Systems, Inc, and by U.S. Patent 5,538,848, owned by The Perkin-Elmer Corporation.

Trademarks

PrecisionPLUS® is a registered trademark of Primer Design Ltd.

genesig® is a registered trademark of Primer Design Ltd.

oasig® is a registered trademark of Primer Design Ltd.

exsig®Mag is a registered trademark of Primer Design Ltd.

The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG. TaqMan® is a registered trademark of Roche Molecular Systems, Inc., The purchase of the Primer Design Ltd reagents cannot be construed as an authorization or implicit license to practice PCR under any patents held by Hoffmann-LaRoche Inc.