Rev. 1

Monkeypox Virus Multiplex qPCR Detection Kit (MPXVD)

Catalog #RU7178
100 tests

Product Description

Monkeypox virus (MPXV) is a zoonotic virus that causes monkeypox in humans and animals. The enveloped double-stranded DNA virus belongs to the *Orthopoxvirus* genus in the *Poxviridae* family with a genome size of around 190 kb. The *Poxviridae* family also includes vaccinia virus, variola virus, cowpox virus, and several animal-related poxviruses. There are two distinct genetic clades of MPXV: the Central African clade and the West African clade. The Central African clade is thought to be more transmissible and causes more severe disease.

ScienCell's Monkeypox Virus Multiplex Detection Kit (MPXVD) is designed to detect the presence of monkeypox virus in dry swab specimen samples or extracted DNA samples from wet swab specimen, cell lysate, body fluid and other biological specimen samples. The cell lysis buffer and enhancer (Cat #GQ400a and GQ400b) are included in the kit to extract DNA from dry swab specimen samples for direct qPCR. The multiplex primer/probe set component (Cat #7178-MPP) contains 3 primer/probe sets: MP1-FAM, MP2-FAM, and HR-HEX. Among them, MP1-FAM and MP2-FAM target highly conserved regions of monkeypox viruses. The carefully

Primer/probe set	Primer/probe target	Probe Reporter Dye
MP1-FAM	Monkeypox virus conserved region 1	FAM
MP2-FAM	Monkeypox virus conserved region 2	FAM
HR-HEX	Human reference gene	HEX

designed MP1-FAM and MP2-FAM primer/probe set can specifically recognize both the Central African clade and the West African clade of MPXV, but will not interact with other *Poxviridae* family viruses, including vaccinia virus, variola virus, and cowpox virus. HR-HEX primer/probe set targets human B2M gene and serves as a control to assess specimen quality. In addition, a non-infectious positive control (Cat #7178-Pos) and nuclease-free water (Cat #MB6048b-1) are included in the kit. The positive control (Cat #7178-Pos) consists of non-infectious viral DNA fragments spiked into human dermal fibroblast cells and serves to ensure reagents and instruments are working properly. Please refer to Tables 4 and 5 for results interpretation.

Kit Components

Cat #	Component	Quantity	Storage
GQ400a	Cell lysis buffer, 10 mL	3 bottles	4°C
GQ400b	Cell lysis buffer enhancer, 100x, 100 µL	3 vials	-20°C
MB6048a-1	SapphiNStart TaqProbe qPCR Master Mix, 2X	1 mL	-20°C
MB6048b-1	Nuclease-free H_2O	1 mL	4°C
7178-MPP	Multiplex primer/probe sets, in solution	600 µL	-20°C
7178-Pos	Positive control (non-infectious; DNA: 1000 – 2000 copies/µL, cells: 400 – 500 counts/µL)	100 µL	-20°C

Additional Materials Required (Materials Not Included in Kit)

Component	Recommended
DNA samples	Customers' samples
DNA isolation kit	ScienCell SpeeDNA Isolation Kit (Cat #MB6918)
qPCR instrument	Multi-channel qPCR instrument supporting FAM and HEX dyes
Heat blocks	With upper temperature limit above 95°C
Microcentrifuge tubes	
qPCR plate or tube	

Quality Control

The primer/probe sets and the positive control are validated by qPCR. The PCR products are analyzed by gel electrophoresis.

Product Use

For Research Use Only. Not for use in diagnostic procedures.

Shipping and Storage

The product is shipped on dry ice. Upon receipt, store the cell lysis buffer enhancer (Cat #GQ400b), SapphiNStart TaqProbe qPCR Master Mix (Cat #MB6048a-1), positive control (Cat #7178-Pos), and Multiplex primer/probe sets (Cat #7178-MPP) at -20°C in a manual defrost freezer. Store the nuclease-free H_2O (Cat #MB6048b-1) and cell lysis buffer (Cat #GQ400a) at 4°C.

Procedures

***Important: Only** use nuclease-free reagents in PCR applications.*

A. Preparation of DNA test samples from dry swab specimen samples

Note: Skip Section A if NOT using dry swab specimen samples.

1. Thaw both the cell lysis buffer enhancer (Cat #GQ400b) and the positive control (Cat #7178-Pos) and keep on ice. After use, put back in a -20°C freezer.

2. Determine the number of dry swab specimen samples (n) and prepare a complete cell lysis buffer mixture.

Reagent	Volume of Reagent Added
Cell lysis buffer (Cat #GQ400a)	(n+1) x 247.5 x 105% µl
Cell lysis buffer enhancer (Cat #GQ400b)	(n+1) x 2.5 x105% µl
Total volume	***(n+1) x 250 x 105% µl***

3. Prepare n+1 microcentrifuge tubes for the samples and a positive control. Dispense 250 µL of complete cell lysis buffer mixture into each tube.

4. Dip each swab in one tube with the complete cell lysis buffer mixture and swirl gently for 10 seconds. Discard the swabs and close the tube caps. For the positive control, add 20 µL of the positive control (Cat #7178-Pos) to one tube with the complete cell lysis buffer mixture, and close the tube cap.

5. Incubate the samples at 55°C for 30 minutes, followed by incubating at 95°C for 10 minutes to fully lyse the samples. Alternatively, transfer 20 µL of each sample from step A.4 to a PCR tube, and run a PCR program as shown in Table 1. Keep lysed samples on ice or store at -20°C.

Table 1. PCR program settings for extracting DNA.

Step	Temperature	Time	Number of cycles
1	55°C	30 min	1
2	95°C	10 min	1
Hold	4°C	Indefinite	1

B. Preparation of DNA test samples from non-dry swab specimen samples

Note: Skip Section B if using dry swab specimen samples.

1. DNA test samples should be prepared using a DNA isolation kit of customer's choice, e.g., ScienCell SpeeDNA Isolation Kit (Cat #MB6918). Please follow the manufacturer's instruction for use to prepare the DNA test samples. A positive control should be prepared together with the test samples by using 20 µL of the positive control (Cat #7178-Pos).

C. qPCR reaction setup

1. Prior to use, allow the multiplex primer/probe sets component (Cat #7178-MPP) to thaw to room temperature in the dark. Shake gently to mix well.

2. Centrifuge the vials at 1,500x g for 1 minute.

3. Aliquot multiplex primer/probe sets as needed. Store at -20°C in a manual defrost freezer. Avoid repeated freeze-and-thaw cycles. Maintain cold and in the dark when thawed.

4. With test samples, two control samples should be run concurrently, the positive control and H_2O (Cat #MB6048b-1) as the No Template Control (NTC). Prepare one 20 µl qPCR reaction as shown in Table 2 for each control sample.

Table 2.

Control sample (positive or NTC)	4 µl
Multiplex primer/probe sets (Cat #7178-MPP)	6 µl
SapphiNStart TaqProbe qPCR Master Mix, 2X (Cat #MB6048a-1)	10 µl
Total volume	***20 µl***

5. For each extracted DNA test sample, prepare one 20 µl qPCR reaction as shown in Table 3.

Table 3.

DNA test sample (concentration varies)	4 µl
Multiplex primer/probe sets (Cat #7178-MPP)	6 µl
SapphiNStart TaqProbe qPCR Master Mix, 2X (Cat #MB6048a-1)	10 µl
Total volume	***20 µl***

6. Seal the qPCR reaction wells. Centrifuge the plates or tubes at 1,500x g for 15 seconds.

7. Setup qPCR reactions as shown in Table 4.

Table 4. Instrument settings for qPCR reactions. Fluorescence data for both FAM and HEX channels should be collected during the data acquisition step.

Step	Temperature	Time	Number of cycles
Enzyme activation	95°C	10 min	1
Denaturation	95°C	20 sec	38
Annealing and	61°C	20 sec	
Extension	72°C	20 sec	
Data acquisition	Plate read, detector (both FAM and HEX)		

Results Interpretation

Table 5. MPXVD kit control sample test results interpretation. A Cq value <35 is considered positive. A Cq value not detected or > 35 is considered negative.

Sample	FAM	HEX	Results Interpretation
7178-Pos	+	+	Expected
	-	-	PCR failed
NTC	-	-	Expected
	If anyone of two is positive		Reagent(s) contaminated

Table 6. MPXVD kit target sample test results interpretation when control results are as expected. A Cq value >35 is considered negative. A Cq value not detected or > 35 is considered negative.

FAM	HEX	Results Interpretation
+	±	MPXV detected
-	+	MPXV not detected
-	-	Invalid result; Check sample quality and repeat the test