December 2022

Monkeypox Virus CDC Probe PCR Assay Protocol Sheet

qPCR Protocol

Sample to Insight

General Information

The Monkeypox Virus (MPXV)-CDC Assays are intended for molecular biology applications for epidemiological research using real-time PCR. The MPXV-CDC Assays are based on published CDC assay designs (1). These assays use hydrolysis probes to detect the monkeypox virus and to differentiate between Congo Basin (CB, renamed as Clade I) and West African (WA, renamed as Clade II) monkeypox strains. The WA assay targets the TNF receptor gene at the terminal inverted repeat region, whereas the CB Assay detects the complement binding protein (C3L) gene. The RNase P Assay targets the human RNase P gene and serves as a sampling- and in-process control.

Upon receipt the product should be stored protected from light at 2–8°C for short-term storage or at –30 to –15°C for long-term storage.

Further information

- *QIAamp® MinElute Virus Vacuum Handbook*: **www.qiagen.com/1063024**
- *QIAamp MinElute Virus Spin Handbook*: **www.qiagen.com/HB-0323**
- *QIAamp DNA Blood Midi/Maxi Handbook*: **www.qiagen.com/HB-0339**
- *QIAamp DNA Blood Mini Handbook*: **www.qiagen.com/HB-0239**
- *QuantiNova® Probe PCR Handbook*: **www.qiagen.com/HB-2699**
- *QuantiNova Multiplex PCR Kit Handbook*: **www.qiagen.com/HB-2105**
- *QuantiNova Pathogen + IC Handbook*: **www.qiagen.com/HB-2147**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- **DNA extraction**: Acceptable specimens for DNA extraction are lesion material and skin biopsies (scab, vesicle roof, or lesion fluid on a dry swab). For DNA extraction the following kits are recommended:

  - QIAamp MinElute Virus Spin Kit (cat. no. 57704)
  - QIAamp MinElute Virus Vacuum Kit (cat. no. 57714)
  - QIAamp DNA Blood Mini Kit (cat. nos. 51104 and 51106)

  The spin column kits can be automated on the QIAcube® Connect and QIAcube Connect MDx. For use with the EZ1® and EZ2® Connect, the EZ1&2™ Virus Mini Kit v2.0 (cat.no. 955134) can be utilized.

- **PCR**: For real-time PCR-based detection of Monkeypox virus DNA in samples the following kits are recommended:

  - QuantiNova Multiplex PCR Kit (cat. nos. 208452, 208454, and 208456)
  - Alternatively, QuantiNova Pathogen Kit (cat. no. 208652 or 208654), QuantiNova Probe PCR Kit (cat. no. 208252, 208254, 208256, or 208257), and the corresponding QuantiTect® kits can be used according to cycling conditions in the individual handbooks.

Procedure

Reaction setup

1. Thaw PCR master mix, DNA, primers, probes, and RNase-free water. Mix the individual solutions.
2. Create a 20x working stock solution of primers and probes for each assay according to Table 1.

Table 1. Setup of 20x primer–probe mix (PPM)

Component	Volume (µL)	Final concentration (µM)
RNase-free water	80	
Forward Primer (100 µM)	8	8
Reverse Primer (100 µM)	8	8
Probe (100 µM)	4	4
Total volume	**100**	

3. Prepare a reaction mix according to Table 2. Due to the hot-start of the PCR reactions, it is not necessary to keep samples on ice during reaction setup or while programming the real-time cycler.

Table 2. Reaction mix setup (96-well PCR plate)

Component	QuantiNova Multiplex PCR Kit	
	Volume	**Final concentration**
4x QuantiNova Multiplex PCR master mix	5 µL	1x
20x PPM	1 µL	0.4 µM primer 0.2 µM probe **Note**: For optimal performance, doubling of the RNase P probe concentration is recommended
RNase-free water	Variable	
DNA	Variable	≤800 ng/reaction
Total volume	**20 µL**	

Note: For ROX depending instruments, please refer to the kit handbook.

4. Vortex the reaction mix. Dispense appropriate volumes of the reaction mix into the wells of a standard PCR plate and spin the plate briefly.
5. Program the real-time cycler according to Table 3.

Note: Data acquisition should be performed during the annealing/extension step in channels according to selected dyes.

Table 3. Cycling conditions for QuantiNova Multiplex PCR Kit

Step	Temperature (°C)	Time	Ramp rate	Number of cycles
Initial heat activation	95	2 min	Maximal/fast mode	1
Denaturation	95	5 s	Maximal/fast mode	40
Annealing/extension	60	30 s	Maximal/fast mode	

Ordering information: qPCR assays

Assays can be ordered via GeneGlobe ID (GG-ID) as individual components (Table 4), combined primer set (Table 5), or complete product (Table 6).

Table 4. Individual components (2x primer and 1x probe)

	Cat. no.	GG-ID	Component(s)	Suggested dye for multiplexing*
1_MPXV-CDC_WA-f	338323	CQM00005	1 Primer	
1_MPXV-CDC_WA-r	338323	CQM00006	1 Primer	
1_MPXV-CDC_WA	338320	CQP00035	Probe	FAM
1_MPXV-CDC_CB-f	338323	CQM00007	1 Primer	
1_MPXV-CDC_CB-r	338323	CQM00008	1 Primer	
1_MPXV-CDC_CB	338320	CQP00034	Probe	Cy5 or ROX
1_RNAse-P(g)_f	338323	CQM00003	1 Primer	
1_RNAse-P(g)_r	338323	CQM00004	1 Primer	
1_Rnase-P(g)	338320	CQP00036	Probe	HEX

*Please find alternative dyes in the Configuration guide below.

Table 5. Combined primer set (pooled primers and probe)

	Cat. no.	GG-ID	Component(s)	Suggested dye for multiplexing*
1_MPXV-CDC_WA	338322	CQR00035	2 Primers	
1_MPXV-CDC_WA	338320	CQP00035	Probe	FAM
1_MPXV-CDC_CB	338322	CQR00034	2 Primers	
1_MPXV-CDC_CB	338320	CQP00034	Probe	Cy5 or ROX
1_RNase-P(g)	338322	CQR00036	2 Primers	
1_RNase-P(g)	338320	CQP00036	Probe	HEX

*Please find alternative dyes in the Configuration guide below.

Table 6. Complete assay (pooled primers and probe)

	Cat. no	GG-ID	Component(s)	Suggested dye for multiplexing*
1_MPXV-CDC_WA	338324	CQB00032	2 Primers + Probe	FAM
1_MPXV-CDC_CB	338324	CQB00031	2 Primers + Probe	Cy5 or ROX
1_RNase-P(g)	338324	CQB00033	2 Primers + Probe	HEX

*Please find alternative dyes in the Configuration guide below.

Configuration guide

Please send the final order with the configuration key(s) to the respective recipient:

Americas: NAGenomicsSupport@qiagen.com
Europe, Middle East, Africa: GS.EMEA@qiagen.com
Rest of World: Genomic.Services@qiagen.com

1. Select the assay and find corresponding catalog number and GG-ID.
2. Select a dye where applicable from Table 7 (for individual probe or the complete assay).
3. Select the scale: 400 or 4000 reactions.
4. Apply the suffix "MP" for monkey pox virus assay.
5. Final assay configuration: for example, CQB00032-F4000MP corresponds to complete assay 1_MPXV-CDC_WA with FAM in 4000-reaction scale

Note: Assays should be ordered with different dyes for multiplexing, for example, Assay1 (FAM) + Assay2 (CY5) + Assay3 (HEX).

Table 7. Dye key for ordering

Dye	FAM	TET	JOE	YAKYE	HEX	CY3	ATTO550	TAMRA	ROX	TxRed	CY35	ATTO647N	CY5	CY55	ATTO680
Key	F	T	J	Y	H	D	A	M	R	X	E	B	C	G	I

References

1. Li, Y., Zhao H., Wilkins K., Hughes, C., and Damon I.K. (2010) Real-time PCR assays for the specific detection of monkeypox virus West African and Congo Basin strain DNA. J. Virol. Methods **169**, 223–227.

Revision history

Revision	Description
August 2022	Initial release
December 2022	Removed epidemiological research using digital PCR in the intended use portion of the General Information section. Updated Tables 1 to 3.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN® kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.