Promega

Product Application

Amplification of Monkeypox DNA with the GoTaq® Endure qPCR Master Mix on the Bio-Rad CFX Opus Real-Time PCR System

Amplify monkeypox DNA standards with the GoTaq® Endure qPCR Master Mix using the Bio-Rad CFX Opus Real-Time PCR System.

Kit: GoTaq® Endure qPCR Master Mix (Cat.# A6220)

Analyses: qPCR

Sample Type(s): Monkeypox Viral DNA

Materials Required:

- GoTaq® Endure qPCR Master Mix (Cat.# A6220)
- Bio-Rad CFX Opus 96 Real-Time PCR System (Bio-Rad, Cat.# 12011319)

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

For further information, see Technical Manual TM752, available at: **www.promega.com/protocols**

or contact Technical Services at: **techserv@promega.com**

Protocol:

1. Prepare the probe qPCR reaction mix according to the table below.

Reagent	Vol/Rxn	# of Reactions	+10% Overage	Total Vol.	Final Conc.
GoTaq® Endure Master Mix, 2X	10µl	x (____)	x 1.1		1X
40X Monkeypox (MPXV) Primer/Probe Mix[1]	0.5µl				1X (500nM Forward and Reverse Primers, 250nM FAM-labeled Probe)
40X RNase P Primer/Probe Mix[1]	0.5µl				1X (250nM Forward and Reverse Primers, 125nM Cy5-labeled Probe)
Nuclease-Free Water	____ to 20µl total				--
Sample	[Xµl]			--	--

2. Add the appropriate volume of the qPCR reaction mix and sample to the PCR plate. Seal the plate with an optical seal.
3. Place the PCR plate in the Bio-Rad CFX Opus 96 Real-Time PCR Instrument, and run the following thermalcycling protocol:

Step	Temperature	Time	# Cycles
Initial Denaturation	95°C	2 minutes	1
Denaturation	95°C	3 seconds	40
Annealing/Extension (with read)	63°C	30 seconds	

4. Analyze the data using the CFX Maestro software with default baseline and threshold settings.

Results:

Monkeypox viral DNA standards and an RNase P control were amplified in a multiplexed reaction with the GoTaq® Endure qPCR Master Mix on the Bio-Rad CFX Opus 96 Real-Time PCR System.

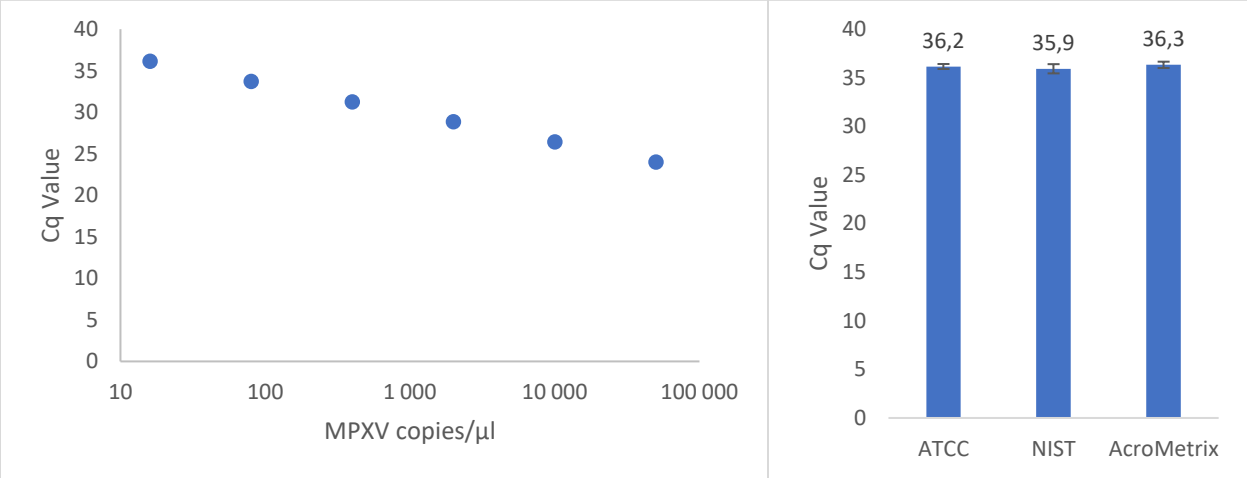

Figure 1. Amplification of monkepox viral DNA standards. Monkeypox standards were serially diluted 1:5 in TE-4 buffer containing 20µg/ml glycogen from 50,000 copies/µl to 16 copies/µl and amplified as described above with the GoTaq® Endure qPCR Master Mix containing K562 genomic DNA spiked at a final concentration of 1ng/µl. 2µl of each standard was amplified in a total reaction volume of 20µl. In the left panel, representative data for a standard curve prepared with the ATCC Quantitative Synthetic Monkeypox virus DNA (ATCC, Cat.# VR-3270SD) is shown, reporting average Cq value ± standard deviation at each concentration (n=3). For the data shown, a linear curve was fit to the data ($log_{10}(concentration)$ versus Cq) with R^2 = 0.997 and a calculated amplification efficiency of 94.8%. For 1ng/µl K562 DNA amplified with RNase P primers/probe in multiplex with the MPXV primers/probe, average Cq value ± standard deviation = 30.76 ± 0.16 (n=24). Similar results were observed with the other DNA standards (data not shown), including the Acrometrix Monkeypox Control 1 (Life Technologies, Cat.# 902050) and the MPXV (Monkeypox) Synthetic DNA PCR Standards (NIST, Cat.# RGTM 10223). The right panel shows the average ± standard deviation of the amplification Cq value for each of the standards diluted to 16 copies/µl (n=6), demonstrating that the three standards result in similar quantitative data.

References:

1. Centers for Disease Control and Prevention. (2022) Test Procedure: Monkeypox virus Generic Real-Time PCR Test, Rev. 01. Retrieved from: https://stacks.cdc.gov/view/cdc/119661.