Certificate of Analysis for NR-9351

Monkeypox Virus Hemagglutinin Gene-Specific Quantitative PCR Assay Detection Kit

Catalog No. NR-9351

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Product Description: The Monkeypox Virus Hemagglutinin (HA) Gene-Specific Quantitative PCR Assay Detection Kit (NR-9351) is designed to detect and quantitate the presence of monkeypox virus. The assay was developed using the hemagglutinin gene from monkeypox virus, Zaire 79 (NR-2324) and consists of the following components:

1) Probe designed with 6-carboxyfluorescein (6-FAM) at the 5' end and both the minor groove binder (MGB) and a non-fluorescent quenching dye at the 3'end (NR-9347)
2) Forward and reverse primers (NR-9348 and NR-9349, respectively)
3) Linearized plasmid-based standard containing an HA gene insert derived from monkeypox virus, Zaire 79 in a commercial vector (NR-4076)

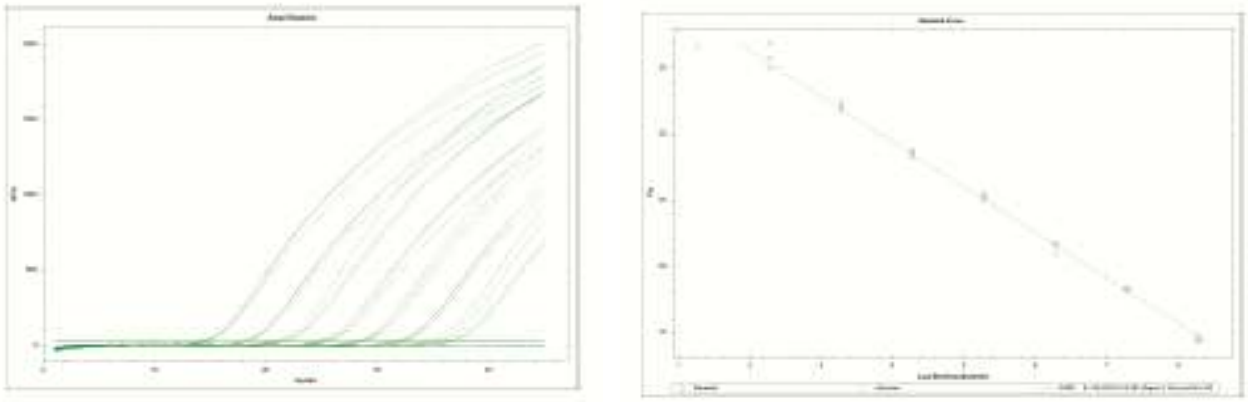
Lot: 61823487

Table 1 – Quantitative Assay

TEST	SPECIFICATIONS	RESULTS
Quantitative PCR – Representative Standard Curve[1]		
Correlation coefficient	~ 0.98	0.992
PCR efficiency	90 to 105%	95.5
Dilution separations (C_T values)	~ 3.3 cycles	~ 3.4 cycles
Quantitative sensitivity	Report results	~ 50 molecules per reaction

[1]See Figure 1.

Figure 1



(A) **(B)**

Representative quantitative PCR cycle graph (A) and associated standard curve (B) using serially diluted NR-4076. The cycle threshold (C_T) was generated using the maximum correlation coefficient approach. Per-well baseline cycles have been determined automatically. The data analysis window is set at 95% of a cycle, centered at the end of the cycle.

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Table 2 – Probe (NR-9347, lot 61694551; Manufactured 04JUN2013)

TEST	SPECIFICATIONS	RESULTS
Content (pmol)	Report results	500
Concentration (µM)	Report results	5

Table 3 – Forward and Reverse Primers

(NR-9348 and NR-9349, lots 61694554 and 61694556; Manufactured 04JUN2013)

TEST	SPECIFICATIONS	RESULTS	
		NR-9348 (Forward primer)	**NR-9349 (Reverse primer)**
PCR Amplification and Sequencing[1] NCBI blast of sequence	Monkeypox virus HA gene	Monkeypox virus HA gene	
Specificity	Report results	Monkeypox virus HA gene	
Content (OD_{260})	Report results	0.200	0.190
Content (µg)	Report results	6.1	6.2
Content (pmol)	Report results	1000	1000
Concentration (µM)	Report results	10	10

[1]BEI Resources NR-4076 (Plasmid Containing Hemagglutinin Gene from Monkeypox Virus, Zaire 79, Linearized) was used as template.

Table 4 – Plasmid-Based Standard (NR-4076, lot 61694558; Manufactured 08MAY2013)

TEST	SPECIFICATIONS	RESULTS
Agarose Gel Electrophoresis of Linearized Plasmid DNA[1]	Migrates as a single band at ~ 5,100 bp	Migrates as a single band at ~ 5,100 bp
DNA Concentration by PicoGreen® Measurement	Report results	60 µg per mL (6 µg/100 µL)
Concentration of DNA Molecules	Calculated using PicoGreen® concentration and molecular weight of plasmid	1.08 X 10^{13} molecules per mL (5.40 X 10^{10} molecules per 5 µL)

[1]DNA from monkeypox virus, Zaire 79 (BEI Resources NR-2324, lot 4729797) was extracted using a QIAamp Viral RNA Minikit (QIAGEN 52904). The HA gene was amplified and cloned into a commercial vector. Plasmid DNA was extracted using a Plasmid Plus Maxi Kit (QIAGEN 12963). Purified plasmid DNA was linearized with *Hin*dIII (New England BioLabs, Inc. R0105S).

Date: 27 SEP 2013 **Signature:** Dorothy C. Young

Title: Technical Manager, BEI Authentication or designee

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected by the vendor or by ATCC® to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

Support Provided by NIAID

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APPENDIX I

Quantitative PCR Assay for the Detection and Quantitation of Monkeypox Virus

Recommended Reagents/Equipment

Reagent/Equipment	Source	Catalog #
Monkeypox Virus Hemagglutinin Gene-Specific Quantitative PCR Probe	BEI Resources	NR-9347, lot 61694551
Monkeypox Virus Hemagglutinin Gene-Specific Quantitative PCR Forward Primer	BEI Resources	NR-9348, lot 61694554
Monkeypox Virus Hemagglutinin Gene-Specific Quantitative PCR Reverse Primer	BEI Resources	NR-9349, lot 61694556
Plasmid Containing Hemagglutinin Gene from Monkeypox Virus, Zaire 79, Linearized	BEI Resources	NR-4076, lot 61694558
iTaq DNA Polymerase Kit	Bio-Rad	170-8870
dNTP Mix	Bio-Rad	170-8874
TE, pH 7.0	Ambion®	AM9861
Molecular Grade Water, or equivalent	ATCC®	60-2450
0.2 mL 8-Tube strips Without Caps	BioRad	TBS-0801, TBS-0851
Optical Flat 8-Cap Strips	BioRad	TCS-0803
Real-Time PCR Plates	BioRad	HSP9601, HSP9901, HSP9655
Microseal 'B' Adhesive Seals	BioRad	MSB-1001
CFX96 Real-Time PCR Detection System	BioRad	185-5096

Preparation of Plasmid-Based Standard Curve Samples

Dilution Tube	Volume (µL)	Volume TE, pH 7.0 (µL)	Concentration (Molecules per 5 µL)[1]
Undiluted NR-4076	---	---	5.40×10^{10}
1	5 of undiluted NR-4076	265	1×10^{9}
2	50 of Tube 1	450	1×10^{8}
3	50 of Tube 2	450	1×10^{7}
4	50 of Tube 3	450	1×10^{6}
5	50 of Tube 4	450	1×10^{5}
6	50 of Tube 5	450	1×10^{4}
7	50 of Tube 6	450	1000
8	50 of Tube 7	450	100
9	50 of Tube 8	450	10
10	50 of Tube 9	450	1

[1]See Certificate of Analysis, Table 4.

Reaction Mix[1]

Reagent	Stock Concentration	Volume per Reaction (µL)
Molecular Grade H_20	---	29.25
PCR Buffer	10X	5
$MgCl_2$	50 mM	6.5
dNTP Mix	10 mM each	1
Probe[2,3] - NRC-1328 (NR-9347)	5 µM	1
Forward Primer[2] - NRC-1329 (NR-9348)	10 µM	1
Reverse Primer[2] NRC-1330 (NR-9349)	10 µM	1
iTaq polymerase	5 units per µL	0.25
Nucleic acid sample	---	5
		Total – 50 µL

[1]Reaction mix should be kept on bench-top cooler until ready for use.
[2]Primers and probe are supplied at working stock concentrations.
[3]6-carboxyfluorescein probe must be protected from light at all times.

Cycling Protocol

Cycle	# of Repeats	Step	Conditions
1	1	1	95.0 °C for 1 minute
2	45	1	95.0 °C for 15 seconds
		2	63.0 °C for 15 seconds

Instructions

1. Prepare unknown nucleic acid samples. Samples used in development of this assay included DNA extracted from virus using the Qiagen QIAamp® Viral RNA Mini Kit following the manufacturer's instructions.
2. This assay was developed using Bio-Rad reagents and detection system. Please refer to the CFX96 Real-Time Detection System Instruction Manual for information regarding plate and run setup.
3. When analyzing the data, especially the standard curve, it is important that the PCR efficiency fall somewhere between 90-105% and that the C_T values are separated by approximately 3.3 cycles.

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