Certificate of Analysis for NR-9351

Monkeypox Virus Hemagglutinin Gene-Specific Quantitative PCR Assay Detection Kit

Catalog No. NR-9351
This reagent is the tangible property of the U.S. Government.

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 (NRC-1328; available individually as NR-9347)
2) Forward and reverse primers (NRC-1329 and NRC-1330; available individually as 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)

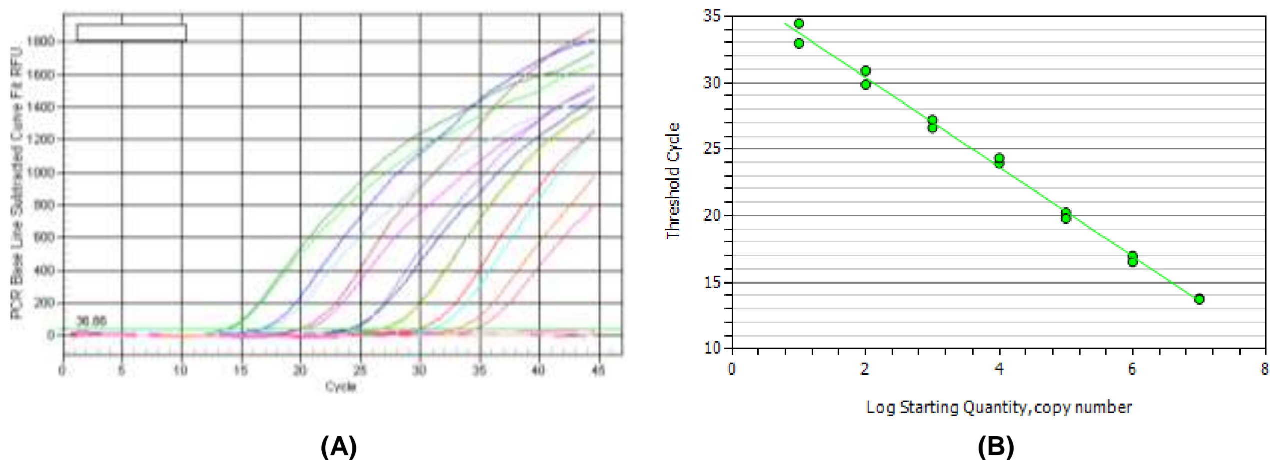
Lot: 58073323

Table 1 – Quantitative Assay

TEST	SPECIFICATIONS	RESULTS
Quantitative PCR – Representative Standard Curve[1]		
Correlation coefficient	~ 0.98	0.995
PCR efficiency	90 to 105%	98.6
Dilution separations (C_T values)	~ 3.3 cycles	~ 3.5 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.

BEI Resources
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Table 2 – Probe (NRC-1328; Manufactured 07SEP2007)

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

Table 3 – Forward and Reverse Primers (NRC-1329 and NRC-1330; Manufactured 06SEP2007)

TEST	SPECIFICATIONS	RESULTS	
		NRC-1329 (Forward primer)	NRC-1330 (Reverse primer)
PCR Amplification and Sequencing[1] Amplicon size NCBI blast of sequence	~190 bp Monkeypox virus HA gene	~190 bp Monkeypox virus HA gene	
Specificity	Report results	Monkeypox virus HA gene	
Content (OD_{260})	Report results	0.193	0.190
Content (µg)	Report results	5.9	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; Manufactured 25AUG2006)

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	1.37 µg per mL (0.14 µg/100 µL)
Concentration of DNA Molecules	Calculated using PicoGreen® concentration and molecular weight of plasmid	2.45×10^{11} molecules per mL (1.225×10^{9} 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 Maxi Kit (QIAGEN 12162). Purified plasmid DNA was linearized with *Hin*dIII (New England BioLabs, Inc. R0105S).

Date: 27 AUG 2013 **Signature:**

Title: Technical Manager, BEI Authentication or designee

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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	NRC-1328 (NR-9347)
Monkeypox Virus Hemagglutinin Gene-Specific Quantitative PCR Forward Primer	BEI Resources	NRC-1329 (NR-9348)
Monkeypox Virus Hemagglutinin Gene-Specific Quantitative PCR Reverse Primer	BEI Resources	NRC-1330 (NR-9349)
Plasmid Containing Hemagglutinin Gene from Monkeypox Virus, Zaire 79, Linearized	BEI Resources	NR-4076
iTaq DNA Polymerase Kit	Bio-Rad	170-8870
dNTP Mix	Bio-Rad	170-8874
TE, pH 7.0	Ambion®	AM9861
Molecular Grade Water	ATCC®	60-2450
0.2 mL 8-Tube strips Without Caps	BioRad	TBS-0201
Optical Flat 8-Cap Strips	BioRad	TCS-0803
iQ Real-Time PCR Plates	BioRad	223-9441
Microseal 'B' Adhesive Seals	BioRad	MSB-1001
iQ5 Multicolor Real-Time PCR Detection System	BioRad	170-9780

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	---	---	1.225×10^{9}
1	44.9 of undiluted NR-4076	505.1	1×10^{8}
2	50 of Tube 1	450	1×10^{7}
3	50 of Tube 2	450	1×10^{6}
4	50 of Tube 3	450	1×10^{5}
5	50 of Tube 4	450	1×10^{4}
6	50 of Tube 5	450	1000
7	50 of Tube 6	450	100
8	50 of Tube 7	450	10
9	50 of Tube 8	450	1

[1]See Certificate of Analysis, Table 4.

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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 iQ5 Multicolor Real-Time PCR Detection System 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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