b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-9350

Pan-Orthopox Virus E9L Gene-Specific Quantitative PCR Assay Detection Kit

Catalog No. NR-9350

This reagent is the tangible property of the U.S. Government.

Product Description: The Pan-Orthopox Virus E9L Gene-Specific Quantitative PCR Assay Detection Kit (NR-9350) is designed to detect and quantitate the presence of orthopox viruses. The assay was developed using a segment of the E9L gene from vaccinia virus, New York City Board of Health (NR-54) 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-1325; available individually as NR-9344)
2) Forward and reverse primers (NRC-1326 and NRC-1327; available individually as NR-9345 and NR-9346, respectively)
3) Linearized plasmid-based standard containing a segment of the E9L gene derived from vaccinia virus in a commercial vector (NRC-1324; available individually as NR-9343)

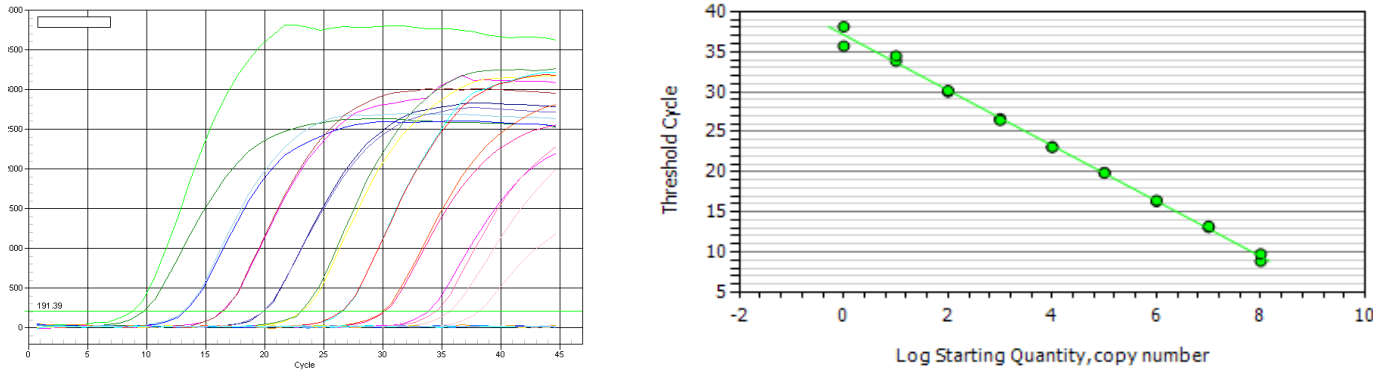
Lot: 63567764

Table 1 – Quantitative Assay

TEST	SPECIFICATIONS	RESULTS
Quantitative PCR – Representative Standard Curve[1]		
Correlation coefficient	~ 0.98	0.997
PCR efficiency	90 to 105%	94.6%
Dilution separations (C_T values)	~ 3.3 cycles	~ 3.5 cycles
Quantitative sensitivity	Report results	10 molecules per reaction

[1]See Figure 1.

Figure 1



(A) **(B)**

Figure 1. Representative quantitative PCR cycle graph (A) and associated standard curve (B) using serially diluted NR-9343. 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 (NRC-1325; Manufactured 07SEP2007)

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

Table 3 – Forward and Reverse Primers (NRC-1326 and NRC-1327; Manufactured 06SEP2007)

TEST	SPECIFICATIONS	RESULTS	
		NRC-1326 (Forward primer)	NRC-1327 (Reverse primer)
PCR Amplification and Sequencing[1] Amplicon size NCBI blast of sequence	~180 bp Orthopox virus E9L gene	~180 bp Orthopox virus E9L gene	
Specificity	Report results	Orthopox virus E9L gene	
Content (OD_{260})	Report results	0.240	0.232
Content (µg)	Report results	7.3	7.3
Content (pmol)	Report results	~ 1000	~ 1000
Concentration (µM)	Report results	10	10

[1]BEI Resources NR-9343 (Plasmid Containing E9L Gene from Vaccinia Virus, NYCBH, Linearized) was used as template.

Table 4 – Plasmid-Based Standard (NRC-1324; Manufactured 09JUL2008)

TEST	SPECIFICATIONS	RESULTS
Agarose Gel Electrophoresis of Linearized Plasmid DNA[1]	Migrates as a single band at ~ 4,100 bp	Migrates as a single band at ~ 4,100 bp
Sequencing of E9L Insert (178 bp)	Orthopox virus E9L gene	Orthopox virus E9L gene Identical to NR-54 sequence
DNA Concentration by PicoGreen® Measurement	Report results	450 ng per mL (45 ng per 100 µL)
Concentration of DNA Molecules	Calculated using PicoGreen® concentration and molecular weight of plasmid	1×10^{11} molecules per mL (5×10^{8} molecules per 5 µL)

[1]DNA from vaccinia virus, NYCBH (BEI Resources NR-54) was extracted using a QIAamp Viral RNA Minikit (QIAGEN 52904). The E9L 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: 19 AUG 2015 **Signature:** Michael R. Gyrla

BEI Resources Authentication

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