

Certificate of Analysis for NR-35510

Rabbitpox Virus Quantitative PCR (qPCR) Assay Detection Kit

Catalog No. NR-35510

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Product Description: The Rabbitpox Virus Quantitative PCR Assay Detection Kit (NR-35510) is designed to detect and quantitate the presence of rabbitpox virus. The assay consists of the following components:

- 1) Probe designed with 6-carboxyfluorescein (6-FAM) at the 5' end and a non-fluorescent quenching dye at the 3' end (NR-26771)
- 2) Forward and reverse primers (NR-26769 and NR-26770, respectively)
- 3) Linearized plasmid-based standard containing rabbitpox virus sequences (NR-26776)

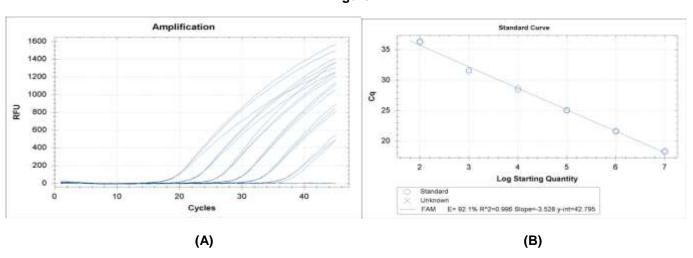
Lot: 61216472

Table 1 - Quantitative Assay

TEST	SPECIFICATIONS	RESULTS
Quantitative PCR – Representative Standard Curve ¹		
Correlation coefficient	~ 0.98	0.99
PCR efficiency	80 to 120%	92.0%
Dilution separations (C _T values)	~ 3.3 cycles	~ 3.5 cycles
Quantitative sensitivity	Report results	100 molecules per reaction

¹See Figure 1

Figure 1



Representative quantitative PCR cycle graph (A) and associated standard curve (B) using NR-35510. The cycle threshold (C_T) was generated using regression analysis. 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 www.beiresources.org E-mail: contact@beiresources.org
Tel: 800-359-7370

Fax: 703-365-2898



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Table 2 – Probe (NR-26771, Lot 60841445; Manufactured 14MAR2012)

TEST	SPECIFICATIONS	RESULTS
Content (pmol)	Report results	1000
Concentration (µM)	Report results	5

Table 3 – Forward and Reverse Primers (NR-26769, Lot 60841442 and NR-26770, Lot 60841444; Manufactured 09MAR2012)

TEST		RESULTS	
	SPECIFICATIONS	NR-26769 (Forward primer)	NR-26770 (Reverse primer)
Content (OD ₂₆₀)	Report results	0.57	0.61
Content (µg)	Report results	19	19
Content (pmol)	Report results	3300	3300
Concentration (µM)	Report results	10	10

¹BEI Resources NR-26776 (Plasmid Containing Rabbitpox Virus Sequences, Linearized) was used as template.

Table 4 – Plasmid-Based Standard (NR-26776, Lot 60841446; Manufactured 15MAY2012)

TEST	SPECIFICATIONS	RESULTS
Agarose Gel Electrophoresis of Linearized Plasmid DNA ¹	Migrates as a single band at ~ 3434 bp	Migrates as a single band at ~ 3434 bp
Concentration of DNA Molecules	Report results	4.27×10^{8} copies per μ L (2.14 × 10 ⁹ copies per 5 μ L)

¹Plasmid DNA was extracted using a Plasmid Midi Kit (QIAGEN 12143). Purified plasmid DNA was linearized with *Eag1-HF* (New England BioLabs, Inc. R3505L).

Date: 27 AUG 2012 Signature: Desothy C. Young

Title: Technical Manager, BEI Authentication or designee

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Fax: 703-365-2898