

Certificate of Analysis for NR-35518

Cowpox Virus Quantitative PCR (qPCR) Assay Detection Kit

Catalog No. NR-35518

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Product Description: The Cowpox Virus Quantitative PCR Assay Detection Kit (NR-35518) is designed to detect and quantitate the presence of cowpox 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 (BHQ-1*plus*) at the 3' end (NR-26774)
- 2) Forward and reverse primers (NR-26772 and NR-26773, respectively)
- 3) Linearized plasmid-based standard containing cowpox virus gene sequences (NR-26775)

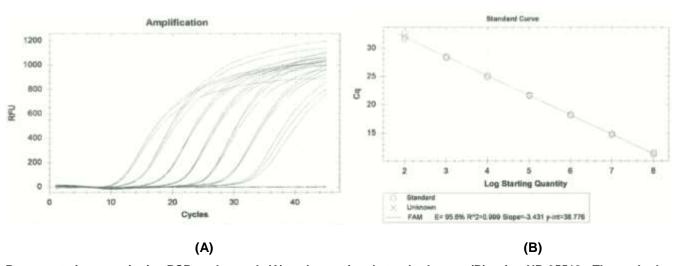
Lot: 61385669

Table 1 - Quantitative Assay

TEST	SPECIFICATIONS	RESULTS
Quantitative PCR – Representative Standard Curve ¹		
Correlation coefficient	~ 0.98	0.99
PCR efficiency	80 to 120%	95.6%
Dilution separations (C _T values)	~ 3.3 cycles	~ 3.4 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-35518. 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.

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Table 2 – Probe (NR-26774, Lot 59776205; Manufactured 06JUN2012)

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

Table 3 – Forward and Reverse Primers (NR-26772, Lot 59776203 and NR-26773, Lot 59776204; Manufactured 22MAY2012)

TEST	SPECIFICATIONS	RESULTS	
		NR-26772 (Forward primer)	NR-26773 (Reverse primer)
Content (OD ₂₆₀)	Report results	0.45	0.47
Content (µg)	Report results	14	15
Content (pmol)	Report results	2000	2000
Concentration (µM)	Report results	10	10

¹BEI Resources NR-26775 (Plasmid Containing Cowpox Virus B9R Gene Sequences, Linearized) was used as template.

Table 4 – Plasmid-Based Standard (NR-26775, Lot 60861982; Manufactured 27JUL2012)

TEST	SPECIFICATIONS	RESULTS
Agarose Gel Electrophoresis of Linearized Plasmid DNA ¹	Migrates as a single band at ~ 3422 bp	Migrates as a single band at ~ 3422 bp
Concentration of DNA Molecules	Report results	2.0×10^8 copies per μ L $(1.0 \times 10^9$ 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. R3505S).

Date: 15 JUL 2013 Signature: Miller & Comple

Title: Technical Manager, BEI Authentication or designee

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