

## **Certificate of Analysis for NR-88**

## Cowpox Virus, Brighton Red

Catalog No. NR-88

(Derived from ATCC® VR-302™)

**Product Description:** Cell lysate and supernatant from African green monkey kidney (BS-C-1) cells<sup>1</sup> infected with cowpox virus, Brighton Red.

Lot<sup>2</sup>: 3694113 Manufacturing Date: 10AUG2004

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in BS-C-1 Cells <sup>1</sup>	Cell rounding and lysis	Cell rounding and lysis
PCR Amplification of Species-Specific Sequence <sup>3</sup>	Cowpox virus	Cowpox virus
Titer by TCID <sub>50</sub> Assay <sup>4,5</sup> in BS-C-1 Cells <sup>1</sup>	Report results	2.5 X 10 <sup>7</sup> TCID <sub>50</sub> /mL
Sterility (21-day incubation)  Harpo's HTYE broth <sup>6</sup> , 37°C and 26°C, aerobic Trypticase soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic Sheep blood agar, 37°C, aerobic Sheep blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic DMEM with 10% FBS, 37°C and 5% CO <sub>2</sub>	No growth	No growth
Mycoplasma Contamination  Agar and broth culture (14-day incubation at 37°C)  DNA Detection by PCR of extracted NR-88 nucleic acid	None detected None detected	None detected None detected

<sup>&</sup>lt;sup>1</sup>BS-C-1 cells: ATCC<sup>®</sup> CCL-26™.

Date: 13 JUN 2006 Signature: Signature on File

> Title: Technical Manager, BEI Authentication

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected 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.

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<sup>&</sup>lt;sup>2</sup>Grown in Eagle's Minimum Essential Media containing Earle's salts and non-essential amino acids (ATCC<sup>®</sup> 30-2003<sup>™</sup>) supplemented with 2% fetal bovine serum (ATCC<sup>®</sup> 30-2021<sup>™</sup>) for 4 days at 37°C and 5% CO<sub>2</sub>.

<sup>3</sup>Ropp, S. L., et al. "PCR Strategy for Identification and Differentiation of Smallpox and other Orthopoxviruses." J. Clin. Microbiol. 33 (1995): 2069–

<sup>2076.</sup> PubMed: 7559950.

<sup>&</sup>lt;sup>4</sup>The Tissue Culture Infectious Dose 50% (TCID<sub>50</sub>) endpoint is the 50% infectious endpoint in cell culture. The TCID<sub>50</sub> is the dilution of virus that under the conditions of the assay can be expected to infect 50% of the culture vessels inoculated, just as a Lethal Dose 50% (LD<sub>50</sub>) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the TCID50 provides a measure of the titer (or infectivity) of a virus preparation.

<sup>&</sup>lt;sup>5</sup>11 days at 37°C and 5% CO₂ with media overlay.

<sup>&</sup>lt;sup>6</sup>Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.