

**Japanese Encephalitis Virus, Nakayama**

**Catalog No. NR-90**

(Derived from ATCC® VR-74™)

**Product Description:** Cell lysate and supernatant from African green monkey kidney (Vero) cells<sup>1</sup> infected with Japanese encephalitis virus (JEV), Nakayama.<sup>2</sup>

**Lot<sup>3</sup>: 57774148**

**Manufacturing Date: 02OCT2007**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero Cells <sup>1</sup>	Report results	Cell rounding and sloughing
Identification by Indirect Fluorescent Antibody Assay <sup>4</sup>	Fluorescence observed	Fluorescence observed
Sequencing of JEV Specific Sequence (~ 1030 bp)	Consistent with JEV	Consistent with JEV
Titer by TCID <sub>50</sub> Assay <sup>5,6</sup> in Vero Cells <sup>1</sup>	Report results	8.9 X 10 <sup>7</sup> TCID <sub>50</sub> /mL
RT-PCR Assay of Extracted RNA Using JEV Specific Primers	~ 1145 bp amplicon	~ 1145 bp amplicon
<b>Sterility (21-day incubation)</b> Harpo's HTYE broth <sup>7</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 No growth No growth No growth No growth No growth	No growth No growth No growth No growth No growth No growth No growth
<b>Mycoplasma Contamination</b> Agar and broth culture (14-day incubation at 37°C) DNA Detection by PCR of Test Article nucleic acid	None detected None detected	None detected None detected

<sup>1</sup>Vero cells: ATCC® CCL-81™.

<sup>2</sup>The inoculum for NR-90 was ATCC® VR-74™ (Lot: 217347).

<sup>3</sup>Grown in Minimum Essential Medium containing Earle's salts and non-essential amino acids (Invitrogen™ 10370-021) supplemented with 2% irradiated fetal bovine serum (Cambrex® 14-471F), 2 mM L-glutamine (Invitrogen™ 25030), and 1 mM sodium pyruvate (Invitrogen™ 11360) for 4 days at 37°C and 5% CO<sub>2</sub>.

<sup>4</sup>Using monoclonal antibody reactive with JEV (Millipore MAB8743).

<sup>5</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 TCID<sub>50</sub> provides a measure of the titer (or infectivity) of a virus preparation.

<sup>6</sup>5 days at 37°C and 5% CO<sub>2</sub>.

<sup>7</sup>Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

**Date:** 10 MAR 2008

**Signature:** Signature on File

**Title:** Technical Manager, BEI Authentication or designee

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