

## Japanese Encephalitis Virus, Okayama *Culex tritaeniorhynchus* (OCT)-541, Line 35-24

### Catalog No. NR-9565

(Derived from ATCC® VR-343™)

**Product Description:** Cell lysate and supernatant from African green monkey kidney (Vero) cells<sup>1</sup> infected with Japanese encephalitis virus (JEV), OCT-541, line 35-24.

**Lot<sup>2</sup>: 58142510**

**Manufacturing Date: 29MAR2008**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero Cells <sup>1</sup>	Report results	Cell rounding and sloughing (Figure 1)
Identification by Indirect Fluorescent Antibody Assay <sup>3,4</sup>	Fluorescence observed	Fluorescence observed
Sequencing of JEV Specific Region (~ 960 bp)	Consistent with JEV	Consistent with JEV
Titer by TCID <sub>50</sub> Assay <sup>4,5</sup> in Vero Cells <sup>1</sup>	Report results	2.8 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>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 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>NR-9565 was produced by inoculation of Vero cells with ATCC® VR-343™ (Lot: 215629) and incubation 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™ 25030-081), and 1 mM sodium pyruvate (Invitrogen™ 11360-070) for 8 days at 30°C and 5% CO<sub>2</sub>.

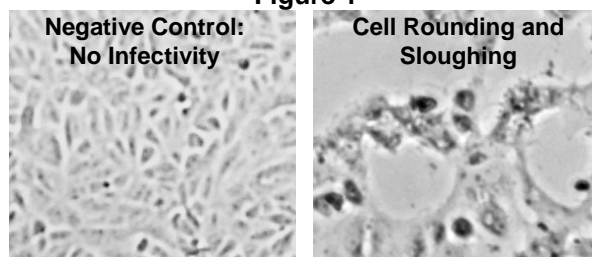
<sup>3</sup>Using monoclonal antibody reactive with JEV (Millipore MAB8743)

<sup>4</sup>14 days at 30°C and 5% CO<sub>2</sub>

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

**Figure 1**



**Date:** 30 July 2008

**Signature:** Signature on file

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

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