

**Dengue Virus Type 4, H241 (Tissue culture-adapted)**

**Catalog No. NR-86**

(Derived from ATCC® VR-1490™)

**Product Description:** Cell lysate and supernatant from Rhesus monkey kidney (LLC-MK2 derivative) cells<sup>1</sup> infected with dengue virus type 4 (DEN-4), H241 (tissue culture-adapted).<sup>2</sup>

**Lot<sup>3</sup>: 57856815**

**Manufacturing Date: 05OCT2007**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in LLC-MK2 Cells <sup>1</sup>	Report results	Cell rounding and degeneration
Identification by Indirect Fluorescent Antibody Assay <sup>4</sup>	Fluorescence observed	Fluorescence observed
Sequencing of DEN-4 Specific Region (~ 405 bp)	Consistent with DEN-4	Consistent with DEN-4
Titer by TCID <sub>50</sub> Assay <sup>5,6</sup> in LLC-MK2 Cells <sup>1</sup>	Report results	1.6 x 10 <sup>5</sup> TCID <sub>50</sub> /mL
RT-PCR Assay of Extracted RNA Using DEN Specific Primers <sup>7</sup>	~ 511 bp amplicon	~ 511 bp amplicon
<b>Sterility (21-day incubation)</b> Harpo's HTYE broth <sup>8</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>LLC-MK2 derivative cells: ATCC® CCL-7.1™

<sup>2</sup>The inoculum for NR-86 was ATCC® VR-1490™ (Lot 1646320).

<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® 14-471E), 2 mM L-glutamine (Invitrogen™ 25030-081), and 1 mM sodium pyruvate (Invitrogen™ 11360-070) for 8 days at 37°C with 5% CO<sub>2</sub>.

<sup>4</sup>Using monoclonal antibody specific to DEN (Millipore MAB8705).

<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>10 days at 37°C with 5% CO<sub>2</sub>.

<sup>7</sup>D1-Lanciotti and D2-Lanciotti primers; Lanciotti, R. S., et al. "Rapid Detection and Typing of Dengue Viruses from Clinical Samples by Using Reverse Transcriptase-Polymerase Chain Reaction." *J. Clin. Microbiol.* 30 (1992): 545-551. PubMed: 1372617.

<sup>8</sup>Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

**Date:** 12 MAR 2008

**Signature:** Signature on File

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

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