

**Dengue Virus Type 2, DakArA1247**

**Catalog No. NR-12221**

**Product Description:** Cell lysate and supernatant from *Aedes albopictus* clone C6/36 cells<sup>1</sup> infected with dengue virus type 2 (DEN-2), DakArA1247.

**Lot<sup>2</sup>: 58526840**

**Manufacturing Date: 15JUN2009**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in C6/36 Cells <sup>1</sup>	Report results	Cell rounding
Identification by Indirect Fluorescent Antibody (IFA) Assay <sup>3</sup>	Fluorescence observed	Fluorescence observed
Sequencing of DEN-2 Specific Sequence (~ 900 nucleotides)	Identical to GenBank EF105383 (DEN-2, DakArA1247)	Identical to GenBank EF105383 (DEN-2, DakArA1247) <sup>4</sup>
Titer by TCID <sub>50</sub> Assay in C6/36 Cells With IFA Readout <sup>1,5,6</sup>	Report results	8.9 x 10 <sup>7</sup> TCID <sub>50</sub> /mL
Functional Activity by RT-PCR Assay Using DEN-2 Specific Primers	~ 1200 bp amplicon	~ 1200 bp amplicon
<b>Bacterial Sterility (BacT/ALERT<sup>®</sup> 3D Microbial Detection System)</b> 14-day incubation of NR-12221: i NST culture bottle, 32°C, anaerobic i AST culture bottle, 32°C, aerobic	No growth No growth	No growth No growth
<b>Fungal Sterility (21-day incubation)</b> Harpo's HTYE broth <sup>7</sup> , 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic Sheep blood agar, 37°C and 26°C, aerobic	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>*Aedes albopictus* clone C6/36 cells (ATCC<sup>®</sup> CRL-1660<sup>™</sup>)

<sup>2</sup>DEN-2, DakArA1247 was deposited by Dr. Rebeca Rico-Hesse of the Department of Virology and Immunology, Southwest Foundation for Biomedical Research, San Antonio, Texas. NR-12221 was grown from deposited virus seed in Minimum Essential Medium containing Earle's salts and non-essential amino acids (Invitrogen<sup>™</sup> 10370-021) supplemented with 2% fetal bovine serum (ATCC<sup>®</sup> 30-2020), 2 mM L-glutamine (Invitrogen<sup>™</sup> 25030-081), and 1 mM sodium pyruvate (Invitrogen<sup>™</sup> 11360-070) for 7 days at 28°C with 5% CO<sub>2</sub>

<sup>3</sup>Using monoclonal antibody specific to DEN-2 (Chemicon MAB8702)

<sup>4</sup>Also consistent with other strains/isolates of DEN-2

<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>7 days at 28°C with 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:** 23 APR 2010

**Signature:** Signature on File

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

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