

## Certificate of Analysis for NR-48801

## Dengue Virus Type 4, 703-4

Catalog No. NR-48801

**Product Description:** Cell lysate and supernatant from *Aedes albopictus* mosquito larval clone C6/36 cells<sup>1</sup> infected with dengue virus type 4 (DEN-4), 703-4

Lot<sup>2</sup>: 62819336 Manufacturing Date: 25SEP2014

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity Using C6/36 Cells <sup>1</sup>	Report results	Cell enlargement and detachment
Identification by Indirect Fluorescent Antibody (IFA) Assay <sup>3</sup>	Fluorescence observed	Fluorescence observed
Sequencing of Species-Specific Region (908 nucleotides)	Consistent with DEN-4, 703-4	99% identity with DEN-4, 703-4 (GenBank: AF231726)
Titer by TCID <sub>50</sub> Assay <sup>4,5</sup> in C6/36 Cells <sup>1</sup> with IFA Readout <sup>3</sup>	Report results	$2.8 \times 10^7 \text{ TCID}_{50} \text{ per mL}$
Functional Activity by RT-PCR Assay	~ 1200 bp amplicon	~ 1200 bp amplicon
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 Test Article nucleic acid	None detected None detected	None detected None detected

<sup>&</sup>lt;sup>1</sup>Aedes albopictus clone C6/36 cells (ATCC<sup>®</sup> CRL-1660™)

**Date:** 13 NOV 2014

Signature:

Title: Technical Manager, BEI Authentication or designee

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 Éagle's Minimum Essential Medium containing Earle's Balanced Salt Solution, non-essential amino acids, 2 mM L-glutamine, 1 mM sodium pyruvate and 1.5 g/L of sodium bicarbonate (ATCC<sup>®</sup> 30-2003) supplemented with 2% fetal bovine serum (ATCC<sup>®</sup> 30-2020) for 7 days at 28°C with 5% CO<sub>2</sub>.

<sup>&</sup>lt;sup>3</sup>Using Anti-Dengue Virus Complex Antibody, clone D3-2H2-9-21 (Millipore MAB8705)

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

<sup>&</sup>lt;sup>5</sup>7 days at 28°C and 5% CO<sub>2</sub>

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