

## **Certificate of Analysis for NR-82**

## Dengue Virus Type 1 (DEN-1), Hawaii

Catalog No. NR-82

Derived from ATCC® VR-1254™

**Product Description:** Cell lysate and supernatant from African green monkey kidney (Vero) cells<sup>1</sup> infected with dengue virus type 1 (DEN-1), Hawaii.

Lot<sup>2</sup>: 64218899 Manufacturing Date: 02MAY2016

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero Cells <sup>1</sup>	Report results	Cell rounding and sloughing
Identification by Indirect Fluorescent Antibody (IFA) Assay³	Fluorescence observed	Fluorescence observed
Sequencing of Species-Specific Region (860 nucleotides)	Consistent with DEN-1, Hawaii	99% identity with DEN-1, Hawaii (GenBank: KM204119)
Titer by TCID <sub>50</sub> Assay <sup>4,5</sup> in Vero Cells <sup>1</sup> with IFA Readout <sup>3</sup>	Report results	8.9 x 10 <sup>6</sup> TCID50 per mL
Amplification of Dengue Virus Sequence by RT-PCR	~ 1000 bp amplicon	~ 1000 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>Vero cells: ATCC® CCL-81™

Date: 18 JUL 2016 Signate

**BEI Resources Authentication** 

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<sup>&</sup>lt;sup>2</sup>Grown in Eagle'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® 30-2003) supplemented with 2% fetal bovine serum (ATCC® 30-2020) for 11 days at 33°C with 5% CO<sub>2</sub>.

<sup>&</sup>lt;sup>3</sup>Using Anti-Dengue Virus Type I Antibody (Millipore MAB8701)

<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>5</sup>14 days at 33°C and 5% CO₂

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