

**Dengue Virus Type 3, UIS 114**

**Catalog No. NR-49706**

**Product Description:** Cell lysate and supernatant from *Aedes albopictus* mosquito larval clone C6/36 cells<sup>1</sup> infected with dengue virus type 3 (DEN-3), UIS 114

**Passage History:** V1C1/C3 (Prior to deposit at BEI Resources/BEI Resources); V# = Number of passages in Vero cells; C# = Number of passages in C6/36 cells<sup>2</sup>

**Lot<sup>3</sup>: 63721380**

**Manufacturing Date: 16OCT2015**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity Using C6/36 Cells <sup>1</sup>	Report results	Cell rounding
Identification by Indirect Fluorescent Antibody (IFA) Assay <sup>4</sup>	Fluorescence observed	Fluorescence observed
Sequencing of Species-Specific Region (480 nucleotides)	Consistent with DEN-3	Consistent with DEN-3 <sup>5</sup>
Titer by TCID <sub>50</sub> Assay <sup>6,7</sup> in C6/36 Cells <sup>1</sup> with IFA Readout <sup>8</sup>	Report results	1.6 × 10 <sup>7</sup> TCID <sub>50</sub> per mL
Amplification of Dengue Virus Sequence by RT-PCR	~ 500 bp amplicon	~ 500 bp amplicon
<b>Sterility (21-day incubation)</b> Harpo's HTYE broth <sup>9</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 extracted Test Article nucleic acid	None detected None detected	None detected None detected

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

<sup>2</sup>The first viral passage at BEI Resources was performed by lipofectamine transfection of extracted viral nucleic acid in order to remove contaminating mycoplasma.

<sup>3</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 9 days at 28°C with 5% CO<sub>2</sub>.

<sup>4</sup>Using Anti-Dengue Virus Complex Antibody (Millipore MAB 8705) or Dengue Virus Type 1-4 Monoclonal Antibody (Thermo Fisher Scientific MA1-27093).

<sup>5</sup>Sequence information for DEN-3, UIS 114 is not available in the NCBI database; nucleotide sequence obtained for NR-49706, Lot No. 63721380 is ~ 99% identical to numerous contemporaneous South American DEN-3 strains.

<sup>6</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>7</sup>14 days at 28°C and 5% CO<sub>2</sub>

<sup>8</sup>Using Anti-Dengue Virus Complex Antibody (Millipore MAB 8705)

<sup>9</sup>Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

## Certificate of Analysis for NR-49706

**Date:** 12 MAY 2017

**Signature:** 

BEI Resources Authentication

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