

**Dengue Virus Type 2, DKA 8521**

**Catalog No. NR-49749**

**Product Description:** Cell lysate and supernatant from *Aedes albopictus* mosquito larval clone C6/36 cells<sup>1</sup> infected with dengue virus type 2 (DEN-2), DKA 8521

**Passage History:** C2/C2 (Prior to deposit at BEI Resources/BEI Resources); C# = Number of passages in C6/36 cells<sup>2</sup>

**Lot<sup>3</sup>: 63777133**

**Manufacturing Date: 20JAN2016**

TEST	SPECIFICATIONS	RESULTS
Identification by Indirect Fluorescent Antibody (IFA) Assay <sup>4</sup>	Fluorescence observed	Fluorescence observed
Sequencing of Species-Specific Region (511 nucleotides)	Consistent with DEN-2	Consistent with DEN-2 <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	8.9 × 10 <sup>5</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
<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 virus 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 7 days at 28°C with 5% CO<sub>2</sub>.

<sup>4</sup>Using Anti-Dengue Virus Type II Antibody (Millipore MAB8702)

<sup>5</sup>Sequence information for DEN-2, DKA 8521 is not available in the NCBI database; nucleotide sequence obtained for NR-49749, Lot No. 63777133 is highly similar to several DEN-2 strains isolated in Singapore in 2009 and 2010.

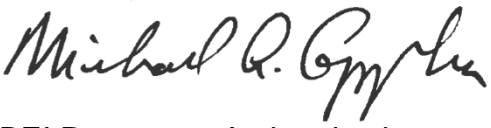
<sup>6</sup>The Tissue Culture Infectious Dose 50% (TCID<sub>50</sub>) endpoint is the 50% infectious endpoint in cell culture. The TCID<sub>50</sub> is the dilution of virus that under the conditions of the assay can be expected to infect 50% of the culture vessels inoculated, just as a Lethal Dose 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>7 days at 28°C and 5% CO<sub>2</sub>

<sup>8</sup>Using Anti-Dengue Virus Complex Antibody (Millipore MAB8705)

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

**Date:** 19 MAY 2016

**Signature:** 

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