

**Dengue Virus Type 4, PR 06-65-740**

**Catalog No. NR-49757**

**Product Description:**

Dengue virus type 4 (DEN-4), PR 06-65-740 was isolated from a human in Puerto Rico in 2006. NR-49757 lot 70058083 was produced by infecting *Aedes albopictus* mosquito larval epithelial cells (clone C6/36; ATCC® CRL-1660™) with BEI Resources lot 64473358 and incubating in Eagle's Minimum Essential Medium (ATCC® 30-2003™) supplemented with 2% fetal bovine serum (ATCC® 30-2020™) for 7 days at 28°C with 5% CO<sub>2</sub>.

**Passage History:**

C(1)/C(3) (Prior to deposit at BEI Resources/BEI Resources); C = C6/36 cells

**Lot: 70058083**

**Manufacturing Date: 01MAR2023**

TEST	SPECIFICATIONS	RESULTS
<b>Identification by Infectivity in C6/36 Cells Confirmed by Indirect Fluorescent Antibody (IFA) Assay<sup>1</sup></b>	Fluorescence observed	Fluorescence observed
<b>Sequencing of Species-Specific Region</b>	Consistent with Dengue virus type 4	Consistent with Dengue virus type 4 <sup>2</sup>
<b>Titer by TCID<sub>50</sub> Assay in C6/36 Cells by Indirect Fluorescent Antibody (IFA) Assay<sup>1,3</sup></b> (7 days at 28°C with 5% CO <sub>2</sub> )	Report results	1.6 × 10 <sup>7</sup> TCID <sub>50</sub> /mL
<b>Sterility (21-day incubation)</b> Harpo's HTYE broth, 37°C and 26°C, aerobic <sup>4</sup> 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, aerobic	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>Using Anti-Dengue Virus Complex Antibody (Millipore MAB 8705)

<sup>2</sup>Sequence information for DEN-4, PR 06-65-740 is not available in the NCBI database; nucleotide sequence obtained for NR-49757 lot 70058083 is ~ 99% identical to numerous DEN-4 strains.

<sup>3</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>4</sup>Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

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18 MAY 2023

Technical Manager or designee, ATCC Federal Solutions

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