Certificate of Analysis for NR-49756

Dengue Virus Type 3, SL 6-14-04

Catalog No. NR-49756

Product Description: Cell lysate and supernatant from *Aedes albopictus* mosquito larval clone C6/36 cells[1] infected with dengue virus type 3 (DEN-3), SL 6-14-04

Passage History: XC1/C3 (Prior to deposit at BEI Resources/BEI Resources); X = Unknown; C# = Number of passages in C6/36 cells[2]

Lot[3]: 70010071 **Manufacturing Date: 08DEC2017**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity Using C6/36 Cells[1]	Report results	Cell rounding and detachment
Identification by Indirect Fluorescent Antibody (IFA) Assay[4]	Fluorescence observed	Fluorescence observed
Sequencing of Species-Specific Region (897 nucleotides)	Consistent with DEN-3	Consistent with DEN-3[5]
Titer by $TCID_{50}$ Assay[6,7] in C6/36 Cells[1] with IFA Readout[4]	Report results	1.6×10^7 $TCID_{50}$ per mL
Amplification of Dengue Virus Sequence by RT-PCR	~ 1000 bp amplicon	~ 1000 bp amplicon
Sterility (21-day incubation)		
Harpo's HTYE broth[8], 37°C and 26°C, aerobic	No growth	No growth
Trypticase soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud broth, 37°C and 26°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, anaerobic	No growth	No growth
Thioglycollate broth, 37°C, anaerobic	No growth	No growth
DMEM with 10% FBS, 37°C and 5% CO_2	No growth	No growth
Mycoplasma Contamination		
Agar and broth culture (14-day incubation at 37°C)	None detected	None detected
DNA detection by PCR of extracted Test Article nucleic acid	None detected	None detected

[1]*Aedes albopictus* clone C6/36 cells (ATCC® CRL-1660™)

[2]The second viral passage at BEI Resources was performed by polyethylenimine (Polyplus-transfection® SA jetPEI® 101-10)-mediated transfection of extracted viral nucleic acid in order to remove contaminating mycoplasma.

[3]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_2.

[4]Using Anti-Dengue Virus Type 3 Envelope Protein Antibody (BEI Resources, NR-15523) or Anti-Dengue Virus Type 3 Antibody (Millipore MAB8703) or Anti-Dengue Virus Complex Antibody (Millipore MAB8705).

[5]Sequence information for DEN-3, SL 6-14-04 is not available in the NCBI database; nucleotide sequence obtained for NR-49756 lot 70010071 is 100% identical to the contemporaneous Sri Lankan dengue isolate DENV3_3060 (GenBank: KX518580; Andrade, C. C., et al. "Rise and Fall of Vector Infectivity During Sequential Strain Displacements by Mosquito-Borne Dengue Virus." J. Evol. Biol. 29 (2016): 2205-2218. PubMed: 27500505) and consistent with numerous other DEN-3 strains.

[6]The Tissue Culture Infectious Dose 50% ($TCID_{50}$) endpoint is the 50% infectious endpoint in cell culture. The $TCID_{50}$ 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_{50}) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the $TCID_{50}$ provides a measure of the titer (or infectivity) of a virus preparation.

[7]7 days at 28°C and 5% CO_2

[8]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

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16 APR 2018

Program Manager or designee, ATCC Federal Solutions

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Support Provided by NIAID

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