

Certificate of Analysis for NR-3786

Dengue Virus Type 1, 228690

Catalog No. NR-3786

Product Description: Cell lysate and supernatant from *Aedes albopictus* clone C6/36 cells¹ infected with dengue virus type 1 (DEN-1), 228690.

Lot^{2,3}: 58613973 Manufacturing Date: 22DEC2009

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in C6/36 Cells ¹	Report results	Cell rounding and syncytia
Identification by Indirect Fluorescent Antibody (IFA) Assay ⁴	Fluorescence observed	Fluorescence observed
Sequencing of DEN-1 Specific Sequence (502 nucleotides)	Consistent with DEN-1	Consistent with DEN-1
Titer by TCID ₅₀ Assay in C6/36 Cells with IFA Readout ^{1,5,6}	Report results	1.6 x 10 ⁷ TCID ₅₀ /mL
Functional Activity by RT-PCR Assay Using DEN Specific Primers	~ 511 bp amplicon	~ 511 bp amplicon
Sterility (21-day incubation) Harpo's HTYE broth ⁷ , 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 ₂	No growth	No growth
Mycoplasma Contamination Agar and broth culture (14-day incubation at 37°C) DNA Detection by PCR of Test Article nucleic acid	None detected None detected	None detected None detected

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

Date: 22 JUN 2010 **Signature:** Signature on File

Title: Technical Manager, BEI Authentication or designee

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²The deposited virus preparation was determined by PCR to be contaminated with *Mycoplasma*. Genomic RNA was extracted from the deposited material and transfected into *Aedes albopictus* clone C6/36 cells. The resulting virus preparation was shown to be free of mycoplasma contamination and was used as the source virus for this lot.

³Grown in Minimum Essential Medium containing Earle's salts and non-essential amino acids (Invitrogen™ 10370-021) supplemented with 2% fetal bovine serum (ATCC ® 30-2020™), 2 mM L-glutamine (Invitrogen™ 25030-081), and 1 mM sodium pyruvate (Invitrogen™ 11360-070) for 7 days at 28°C with 5% CO₂

⁴Using monoclonal antibody specific to dengue complex (Chemicon MAB8705)

⁵The Tissue Culture Infectious Dose 50% (TCID₅₀) endpoint is the 50% infectious endpoint in cell culture. The TCID₅₀ 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% (LD₅₀) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the TCID₅₀ provides a measure of the titer (or infectivity) of a virus preparation.

⁶7 days at 28°C with 5% CO₂

⁷Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.