

Dengue Virus Type 4, BC13/97

Catalog No. NR-3805

Product Description: Cell lysate and supernatant from *Aedes albopictus* clone C6/36 cells¹ infected with dengue virus type 4 (DEN-4), BC13/97.

Lot^{2,3}: 58613989

Manufacturing Date: 12MAY2010

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in C6/36 Cells¹	Report results	Cell aggregation and sloughing
Identification by Indirect Fluorescent Antibody (IFA) Assay⁴	Fluorescence observed	Fluorescence observed
Sequencing of DEN-4 Specific Sequence (956 nucleotides)	Consistent with DEN-4	Consistent with DEN-4
Titer by TCID₅₀ Assay in C6/36 Cells With IFA Readout^{1,5,6}	Report results	1.6 x 10 ⁸ TCID ₅₀ /mL
Functional Activity by RT-PCR Assay Using DEN Specific Primers	~ 1200 bp amplicon	~ 1200 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 No growth No growth No growth No growth No growth	No growth No growth No growth No growth No growth 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

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

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 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% 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. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Date: 07 FEB 2011

Signature: 

Title: Technical Manager, BEI Authentication or designee

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