

Certificate of Analysis for NR-3787

Dengue Virus Type 1, BC89/94

Catalog No. NR-3787

Product Description: Cell lysate and supernatant from *Aedes albopictus* clone C6/36 cells¹ infected with dengue virus type 1 (DEN-1), BC89/94.

Lot^{2,3}: 58613974 Manufacturing Date: 20NOV2009

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in C6/36 Cells ¹	Report results	Cell rounding and enlargement
Identification by Indirect Fluorescent Antibody (IFA) Assay ⁴	Fluorescence observed	Fluorescence observed
Sequencing of DEN-1 Specific Sequence (551 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	8.9 x 10 ⁷ 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
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: 07 FEB 2011

Signature.

Title: Technical Manager, BEI Authentication or designee

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC® knowledge.

ATCC® is a trademark of the American Type Culture Collection.

You are authorized to use this product for research use only. It is not intended for human use.

Biodefense and Emerging Infections Research Resources Repository

www.beiresources.org

E-mail: contact@beiresources.org

Tel: 800-359-7370 Fax: 703-365-2898

NR-3787_58613974_07FEB2011

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 8 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.

⁶9 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.