

Certificate of Analysis for NR-49746

Dengue Virus Type 2, C 0590-94

Catalog No. NR-49746

Product Description: Cell lysate and supernatant from *Aedes albopictus* mosquito larval clone C6/36 cells¹ infected with dengue virus type 2 (DEN-2), C 0590-94

Passage History: X/C2/C3 (Unknown/WRCEVA/BEI Resources); X = Unknown, C# = Number of passages in C6/36 cells

Lot²: 63762276 Manufacturing Date: 17DEC2015

TEST	SPECIFICATIONS	RESULTS
Identification by Indirect Fluorescent Antibody (IFA) Assay ³	Fluorescence observed	Fluorescence observed
Sequencing of Species-Specific Region (454 nucleotides)	Consistent with DEN-2	Consistent with DEN-24
Titer by TCID ₅₀ Assay ^{5,6} in C6/36 Cells ¹ with IFA Readout ⁷	Report results	1.6 × 10 ⁸ TCID ₅₀ per mL
Amplification of Dengue Virus Sequence by RT-PCR	~ 500 bp amplicon	~ 500 bp amplicon
Sterility (21-day incubation) Harpo's HTYE broth ⁸ , 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 extracted Test Article nucleic acid	None detected None detected	None detected None detected

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

Date: 10 MAY 2016

Signature:

BEI Resources Authentication

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®'s knowledge.

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²Grown in Éagle'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.

³Using Anti-Dengue Virus Complex Antibody (Millipore MAB8705)

⁴Only minimal sequence information for DEN-2, C 0590-94 is available in the NCBI database (GenBank: U87357); nucleotide sequence obtained for NR-49746, Lot No. 63762276 is highly similar to DENV-2/ThNH-7/93 (GenBank: AF022434), a DEN-2 strain that was isolated in Thailand in 1993.

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.

⁶6 days at 28°C and 5% CO₂

⁷Using Anti-Dengue Virus Type II Antibody (Millipore MAB8702)

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