

Certificate of Analysis for NR-44085

Dengue Virus Type 2, DENV-2/VN/BID-V1002/2006

Catalog No. NR-44085

Product Description: Cell lysate and supernatant from *Aedes albopictus* mosquito larval clone C6/36 cells¹ infected with dengue virus type 2, DENV-2/VN/BID-V1002/2006

Lot²: 63143139 Manufacturing Date: 18MAR2015

TEST	SPECIFICATIONS	RESULTS
Identification by Indirect Fluorescent Antibody (IFA) Assay ³	Fluorescence observed	Fluorescence observed
Sequencing of Species-Specific Region (1046 nucleotides)	Consistent with DENV-2/VN/BID-V1002/2006	99% identity with DENV-2/VN/BID-V1002/2006 (GenBank: EU482447)
Titer by TCID ₅₀ Assay ^{4,5} in C6/36 Cells ¹ with IFA Readout ³	Report results	5 x 10 ⁶ TCID ₅₀ per mL
Functional Activity by RT-PCR Assay	~ 1400 bp amplicon	~ 1400 bp amplicon
Sterility (21-day incubation) Harpo's HTYE broth ⁶ , 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: 28 MAY 2015 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 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 6 days at 28°C with 5% CO₂.

³Using Anti-Dengue Virus Type II Antibody (Millipore MAB8702)

⁴The 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.

⁵7 days at 28°C and 5% CO₂

⁶Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press. 2004. p. 798.