

Dengue Virus Type 3, PR 06-65-544

Catalog No. NR-49753

Product Description: Dengue virus type 3 (DEN-3), PR 06-65-544 was isolated from a human in Puerto Rico in 2006. Each vial contains cell lysate and supernatant from *Aedes albopictus* mosquito larval clone C6/36 cells¹ infected with DEN-3, PR 06-65-544

Passage History: C1/C2 (Prior to deposit at BEI Resources/BEI Resources); C = C6/36 cells¹

Lot²: 70016040

Manufacturing Date: 31JUL2018

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity Using C6/36 Cells ¹	Report results	Cell rounding and detachment
Identification by Indirect Fluorescent Antibody (IFA) Assay ³	Fluorescence observed	Fluorescence observed
Sequencing of Species-Specific Region (~ 490 nucleotides)	≥ 98% identity with DEN-3	≥ 98% identity with DEN-3 ⁴
Titer by TCID ₅₀ Assay ^{5,6} in C6/36 Cells ¹ with IFA Readout ³	Report results	1.6 × 10 ⁶ TCID ₅₀ per mL
Amplification of Dengue Virus Sequence by RT-PCR	~ 510 base pair amplicon	~ 510 base pair 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 extracted Test Article nucleic acid	None detected None detected	None detected None detected

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

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 7 days at 28°C with 5% CO₂.

³Using Anti-Dengue Virus Type 3 Antibody (Millipore MAB8703)

⁴Sequence information for DEN-3, PR 06-65-544 is not available in the NCBI database. Nucleotide sequence obtained for NR-49753 lot 70016040 is 99.9% identical to the Puerto Rico dengue 3 isolate DENV-3/PR/BID-V1728/2006, complete genome (GenBank: KF955456.1) and consistent with numerous other DEN-3 strains.

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 and 5% CO₂

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

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