

Dengue Virus Type 4, UIS 497

Catalog No. NR-49724

Product Description:

Dengue virus type 4 (DENV-4), UIS 497 was isolated from a serum specimen collected from a human in Santander, Colombia in November 2004. NR-49724 lot 70026347 was produced by infecting *Aedes albopictus* cells (C6/36; ATCC® CRL-1660™) with BEI Resources seed material lot 63721383 and incubating 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 9 days at 28°C with 5% CO₂.

Passage History:

C2/C4 (Prior to deposit at BEI Resources/BEI Resources); C = C6/36 cells

Lot: 70026347

Manufacturing Date: 13JUN2019

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in C6/36 Cells	Report results	Cell rounding and detachment
Identification by Indirect Fluorescent Antibody (IFA) Assay ¹	Fluorescence observed	Fluorescence observed
Sequencing of Species-Specific Region (~ 700 nucleotides)	Consistent with DENV-4	Consistent with DENV-4 ²
Titer by TCID ₅₀ Assay in C6/36 Cells by Fluorescent Antibody Assay ^{1,3} (14 days at 28°C with 5% CO ₂)	Report results	8.9 × 10 ⁷ TCID ₅₀ /mL
Amplification of Dengue Virus Sequence by RT-PCR	~ 1100 base pair amplicon	~ 1100 base pair amplicon
Sterility (21-day incubation) Harpo's HTYE broth, 37°C and 26°C, aerobic ⁴ 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, aerobic	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

¹Using Anti-Dengue Virus Complex Antibody (Millipore MAB 8705)

²Sequence information for DENV-4, UIS 497 is not available in the NCBI database; the nucleotide sequence obtained for NR-49724 lot 70026347 is ≥ 99% identical to DENV-4/CO/BID-V3410/2004 (GenBank: GQ868583.1) and DENV-4/CO/BID-V3411/2004 (GenBank: GQ868584.1), two DENV-4 strains that were also isolated in Santander, Colombia in 2004, and consistent with numerous additional DENV-4 strains²

³The 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.

⁴Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

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03 JUN 2024

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