b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-49724

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.

Passage History:
C1/C4 (Prior to deposit at BEI Resources/BEI Resources); C = C6/36 cells[1]

Lot: 70026347[2,3] **Manufacturing Date: 13JUN2019**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in C6/36 cells[1]	Report results	Cell rounding and detachment
Identification by Indirect Fluorescent Antibody (IFA) Assay[4]	Fluorescence observed	Fluorescence observed
Sequencing of Species-Specific Region (~ 700 nucleotides)	Consistent with DENV-4	Consistent with DENV-4[5]
Titer by $TCID_{50}$ Assay in C6/36 cells by IFA Readout[1,4,6,7]	Report results	8.9×10^7 $TCID_{50}$ per 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[8]	No growth	No growth
Trypticase Soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud broth, 37°C and 26°C, aerobic	No growth	No growth
Blood agar, 37°C, aerobic	No growth	No growth
Blood agar, 37°C, anaerobic	No growth	No growth
Thioglycollate broth, 37°C, anaerobic	No growth	No growth
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)	None detected	None detected
DNA detection by PCR of extracted Test Article nucleic acid	None detected	None detected

[1]*Aedes albopictus* clone C6/36 cells (ATCC® CRL-1660™)
[2]The first virus passage at BEI Resources was performed by lipofectamine transfection of extracted viral nucleic acid to remove contaminating mycoplasma.
[3]NR-49724 lot 70026347 was produced by infecting C6/36 cells with BEI Resources NRS-49724 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% CO_2.
[4]Using Anti-Dengue Virus Complex Antibody (Millipore MAB 8705)
[5]Sequence 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 strains.
[6]The Tissue Culture Infectious Dose 50% ($TCID_{50}$) endpoint is the 50% infectious endpoint in cell culture. The $TCID_{50}$ 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% (LD_{50}) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the $TCID_{50}$ provides a measure of the titer (or infectivity) of a virus preparation.
[7]Assay plates were incubated 14 days at 28°C and 5% CO_2.
[8]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

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/Heather Couch/
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Program Manager or designee, ATCC Federal Solutions

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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