

Certificate of Analysis for NR-49781

St. Louis Encephalitis Virus, V 07457

Catalog No. NR-49781

Product Description:

St. Louis encephalitis virus (SLEV), V 07457 was isolated from a mosquito (*Culex quinquefasciatus*) in Harris County, Texas, USA on August 29, 2013. NR-49781 lot 70014025 was produced by infecting *Cercopithecus aethiops* kidney epithelial cells (Vero; ATCC[®] CCL-81[™]) with the deposited material and incubating in Eagle's Minimum Essential Medium (ATCC[®] 30-2003) supplemented with 2% fetal bovine serum (ATCC[®] 30-2020) for 6 days at 37°C with 5% CO₂. The second virus passage at BEI Resources was performed by polyethylenimine (Polyplus-transfection[®] SA jetPEI[®] 101-10)-mediated transfection of extracted viral nucleic acid in order to remove contaminating mycoplasma.

Passage History:

C6/36(1)V(1)/V(3) (Prior to deposit at BEI Resources/BEI Resources); C6/36 = Aedes albopictus clone C6/36 cells; V = Vero cells

Lot: 70014025 Manufacturing Date: 25APR2019

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TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero Cells	Cell rounding and detachment	Cell rounding and detachment
Sequencing of Species-Specific Region (950 nucleotides)	≥ 98% identity with SLEV	≥ 98% identity with SLEV¹
Titer by TCID₅ Assay in Vero Cells by Cytopathic Effect² (8 days at 37°C with 5% CO2)	Report results	2.8 × 10 ⁷ TCID ₅₀ per mL
Amplification of SLEV Sequence by RT-PCR	~ 1000 base pair amplicon	~ 1000 base pair amplicon
Sterility (21-day incubation)		
Harpo's HTYE broth, 37°C and 26°C, aerobic ³	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
Sheep blood agar, 37°C, aerobic	No growth	No growth
Sheep 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

Sequence information for SLEV, V 07457 is not available in the NCBI database; nucleotide sequence obtained for NR-49781 lot 70014025 is ≥ 98% identical to numerous SLEV strains.

/Heather Couch/ Heather Couch

13 MAY 2020

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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²The 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.
³Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.