

Certificate of Analysis for NR-50061

St. Louis Encephalitis Virus, TX AR 9-6038

Catalog No. NR-50061

Product Description:

St. Louis encephalitis virus (SLEV), TX AR 9-6038 was isolated from a pool of mosquitoes (*Culex quinquefasciatus*) in Port Arthur, Jefferson County, Texas, USA in August 2009. NR-50061 lot 70014031 was produced by infecting *Cercopithecus aethiops* kidney epithelial cells (Vero; ATCC[®] CCL-81[™]) and incubating in Eagle's Minimum Essential Medium (EMEM; ATCC[®] 30-2003) supplemented with 2% fetal bovine serum (ATCC[®] 30-2020) for 7 days at 37°C with 5% CO₂. The second viral 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:

BHK(1)V(1)/V(3) (Prior to deposit at BEI Resources/BEI Resources); BHK = Baby hamster kidney cells; V = Vero cells

Lot: 70014031 Manufacturing Date: 14FEB2019

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero Cells	Cell rounding and detachment	Cell rounding and detachment
Sequencing of Species-Specific Region (~ 980 nucleotides)	Consistent with SLEV	Consistent with SLEV ¹
Titer by TCID₅₀ Assay in Vero Cells by Cytopathic Effect² 10 days at 37°C with 5% CO₂	Report results	8.9 × 10 ⁷ TCID ₅₀ per mL
Amplification of SLEV Sequence by RT-PCR	~ 1020 base pair amplicon	~ 1020 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

¹Sequence information for SLEV, TX AR 9-6038 is not available in the NCBI database; nucleotide sequence obtained for NR-50061 lot 70014031 is ≥ 98% identical to numerous SLEV strains.

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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.



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/Heather Couch/ Heather Couch

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Program Manager or designee, ATCC Federal Solutions

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