b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-49782

St. Louis Encephalitis Virus, V 08449

Catalog No. NR-49782

Product Description:

St. Louis encephalitis virus (SLEV), V 08449 was isolated from a mosquito *(Culex quinquefasciatus)* in Harris County, Texas, USA in August 2013. NR-49782 lot 70014027 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 5 days at 37°C with 5% CO_2. The second virus passage at BEI Resources was performed by polyethylenimine (Polyplus-transfection® SA jetPEI® 101-10N)-mediated transfection of extracted viral nucleic acid in order to remove contaminating mycoplasma.

Passage History:

C6/36(2)V(1)/V(3) (Prior to deposit at BEI Resources/BEI Resources); C6/36 = *Aedes albopictus* clone C6/36 cells; V = Vero cells

Lot: 70014027 **Manufacturing Date: 13MAR2019**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero Cells	Cell rounding and detachment	Cell rounding and detachment
Sequencing of Species-Specific Region (~ 830 nucleotides)	≥ 98% identity with SLEV	≥ 98% identity with SLEV[1]
Titer by $TCID_{50}$ Assay in Vero Cells by Cytopathic Effect[2] (8 days at 37°C with 5% CO_2)	Report results	1.6×10^8 $TCID_{50}$ 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

[1]Sequence information for SLEV, V 08449 is not available in the NCBI database; nucleotide sequence obtained for NR-49782 lot 70014027 is ≥ 98% identical to numerous SLEV strains.
[2]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.
[3]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

/Heather Couch/
Heather Couch 15 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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