b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-49923

West Nile Virus, KERN 5281

Catalog No. NR-49923

Product Description: West Nile virus (WNV), KERN 5281 was isolated from a mosquito *(Culex tarsalis)* in Kern County, California, USA on June 2, 2005. Each vial contains cell lysate and supernatant from *Cercopithecus aethiops* kidney epithelial cells[1] infected with WNV, KERN 5281.

Passage History: V1/V3 (Prior to deposit at BEI Resources/BEI Resources); V = Vero cells[1]

Lot[2,3]: 70004668 **Manufacturing Date: 06NOV2017**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero cells	Cell rounding and detachment	Cell rounding and detachment
Sequencing of Species-Specific Region (~ 760 nucleotides)	Consistent with WNV	Consistent with WNV[4]
Titer by $TCID_{50}$ Assay[5,6] in Vero cells[1] by Cytopathic Effect	Report results	1.6×10^8 $TCID_{50}$ per mL
Amplification of WNV Sequence by RT-PCR	~ 920 base pair amplicon	~ 920 base pair amplicon
Sterility (21-day incubation)		
Harpo's HTYE broth[7], 37°C and 26°C, aerobic	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]Vero: ATCC® CCL-81™
[2]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.
[3]Grown 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 3 days at 37°C with 5% CO_2
[4]Sequence information for WNV, KERN 5281 is not available in the NCBI database; nucleotide sequence obtained for NR-49923 lot 70004668 is 100% identical to numerous WNV strains.
[5]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.
[6]5 days at 37°C and 5% CO_2
[7]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

/Heather Couch/
Heather Couch 10 AUG 2018
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.

Support Provided by NIAID

BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898