

## **Certificate of Analysis for NR-49925**

## West Nile Virus, LA 13-06

Catalog No. NR-49925

**Product Description:** West Nile virus (WNV), LA 13-06 was isolated from a *Cyanocitta cristata* (Blue Jay) in Lafayette Parish, Louisiana, USA, on October 9, 2006. Each vial contains cell lysate and supernatant from *Cercopithecus aethiops* kidney epithelial cells<sup>1</sup> infected with WNV, LA 13-06.

Passage History: V1/V2 (Prior to deposit at BEI Resources/BEI Resources); V = Vero cells<sup>1</sup>

Lot<sup>2</sup>: 70004672 Manufacturing Date: 13JUN2017

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero cells <sup>1</sup>	Cell rounding and detachment	Cell rounding and detachment
Sequencing of Species-Specific Region (~ 750 nucleotides)	Consistent with WNV	99.6% identity with WNV, WNV- 1/Cyanocitta cristata/USA/ 06VER01768/2006 (GenBank: KX547553.1) <sup>3</sup>
Titer by TCID <sub>50</sub> Assay <sup>4,5</sup> in Vero cells <sup>1</sup> by Cytopathic Effect	Report results	2.8 x 108 TCID <sub>50</sub> 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 <sup>6</sup> , 37°C and 26°C, aerobic Trypticase soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic Sheep blood agar, 37°C, aerobic Sheep blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic DMEM with 10% FBS, 37°C and 5% CO <sub>2</sub>	No growth	No growth
Mycoplasma Contamination  Agar and broth culture (14-day incubation at 37°C)  DNA detection by PCR of extracted Test Article nucleic acid	None detected None detected	None detected None detected

¹Vero: ATCC® CCL-81™

## /Heather Couch/

Heather Couch 13 JUL 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.

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BEI Resources www.beiresources.org E-mail: contact@beiresources.org

Tel: 800-359-7370 Fax: 703-365-2898

<sup>&</sup>lt;sup>2</sup>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<sup>®</sup> 30-2003) supplemented with 2% fetal bovine serum (ATCC<sup>®</sup> 30-2020) for 4 days at 37°C with 5% CO<sub>2</sub>

<sup>&</sup>lt;sup>3</sup>There is no sequence information for WNV, LA 13-06 in the NCBI database or any of the various virology databases. The polyprotein gene sequence obtained for NR-49925 is nearly identical to the published sequence of WNV, WNV-1/*Cyanocitta cristata*/USA/06VER01768/2006, a WNV strain isolated from a mosquito pool in Louisiana in 2006 and consistent with numerous WNV strains.

<sup>&</sup>lt;sup>4</sup>The Tissue Culture Infectious Dose 50% (TCID<sub>50</sub>) endpoint is the 50% infectious endpoint in cell culture. The TCID<sub>50</sub> 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<sub>50</sub>) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the TCID<sub>50</sub> provides a measure of the titer (or infectivity) of a virus preparation.

<sup>&</sup>lt;sup>5</sup>4 days at 37°C and 5% CO<sub>2</sub>

<sup>&</sup>lt;sup>6</sup>Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.