

## **Certificate of Analysis for NR-49789**

West Nile Virus, TM 171-03

Catalog No. NR-49789

**Product Description:** Cell lysate and supernatant from *Cercopithecus aethiops* kidney epithelial cells<sup>1</sup> infected with West Nile virus (WNV), TM 171-03

**Passage History:** SMXV2/V3 (Prior to deposit at BEI Resources/BEI Resources); SM# = Number of passages in suckling mice; V# = Number of passages in Vero cells<sup>2</sup>; X = Unknown

Lot<sup>3</sup>: 64498463 Manufacturing Date: 12DEC2016

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero cells	Cell rounding and detachment	Cell rounding and detachment
Sequencing of Species-Specific Region (704 nucleotides)	Consistent with WNV, TM 171-03	100% identity with WNV, TM 171-03 (GenBank: KU978769)
Titer by TCID <sub>50</sub> Assay <sup>4,5</sup> in Vero cells <sup>1</sup>	Report results	8.9 × 10 <sup>7</sup> TCID <sub>50</sub> per mL
Amplification of WNV Sequence by RT-PCR	~ 920 bp amplicon	~ 920 bp 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

<sup>&</sup>lt;sup>1</sup>Vero: ATCC<sup>®</sup> CCL-81 ™

Date: 18 JAN 2018

Signature:

**BEI Resources Authentication** 

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.

ATCC® is a trademark of the American Type Culture Collection.

You are authorized to use this product for research use only. It is not intended for human use.

BEI Resources www.beiresources.org E-mail: <a href="mailto:contact@beiresources.org">contact@beiresources.org</a>
Tel: 800-359-7370

Fax: 703-365-2898

<sup>&</sup>lt;sup>2</sup>The second virus passage at BEI Resources was performed by lipofectamine-mediated transfection of extracted viral nucleic acid in order to remove contaminating mycoplasma.

<sup>&</sup>lt;sup>3</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 3 days at 37°C with 5% CO<sub>2</sub>

<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>56</sup> days at 37°C and 5% CO2

<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.