b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-49796

West Nile Virus, TX 9364 (D0279)

Catalog No. NR-49796

Product Description: Cell lysate and supernatant from *Cercopithecus aethiops* kidney epithelial cells[1] infected with West Nile virus (WNV), TX 9364 (D0279)

Passage History: V1/V3 (Prior to deposit at BEI Resources/BEI Resources); V# = Number of passages in Vero cells[2]

Lot[3]: 64498480 **Manufacturing Date: 27MAR2017**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero cells	Cell rounding and detachment	Cell rounding and detachment
Sequencing of Species-Specific Region (795 nucleotides)	Consistent with WNV	Consistent with WNV[4]
Titer by $TCID_{50}$ Assay[5,6] in Vero cells[1]	Report results	8.9×10^9 $TCID_{50}$ per mL
Amplification of WNV Sequence by RT-PCR	~ 920 bp amplicon	~ 920 bp 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 lipofectamine 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, TX 9364 (D0279) is not available in the NCBI database; nucleotide sequence obtained for NR-49796 lot 64498480 is highly similar 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]7 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.

Date: 04 DEC 2017 **Signature:** Michael R. Gyrla

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