

Certificate of Analysis for NR-49790

West Nile Virus, TX 4090 (D1660)

Catalog No. NR-49790

Product Description: Cell lysate and supernatant from *Cercopithecus aethiops* kidney epithelial cells¹ infected with West Nile virus (WNV), TX 4090 (D1660)

Passage History: V2; V# = Number of passages in Vero cells²

Lot³: 64498464 Manufacturing Date: 01MAR2017

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero cells	Cell rounding and detachment	Cell rounding and detachment
Sequencing of Species-Specific Region (803 nucleotides)	Consistent with WNV	Consistent with WNV ⁴
Titer by TCID ₅₀ Assay ^{5,6} in Vero cells ¹	Report results	1.6 x 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 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 ₂	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™

Date: 02 JAN 2018

lature.

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²The deposited material for WNV, TX 4090 (D1660) was the original brain homogenate from an American crow *(Corvus brachyrhynchos)*.

³Grown 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 5 days at 37°C with 5% CO₂

⁴Sequence information for WNV, TX 4090 (D1660) is not available in the NCBI database; nucleotide sequence obtained for NR-49790 lot 64498464 is highly similar to numerous WNV strains.

⁵The Tissue Culture Infectious Dose 50% (TCID₅₀) endpoint is the 50% infectious endpoint in cell culture. The TCID₅₀ 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% (LD₅₀) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the TCID₅₀ provides a measure of the titer (or infectivity) of a virus preparation.

 $^{^6}$ 8 days at 37°C and 5% CO $_2$

⁷Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.