

Certificate of Analysis for NR-50806

West Nile Virus, TX 9410 (D0325), Gamma-Irradiated

Catalog No. NR-50806

Product Description: Cell lysate and supernatant from *Cercopithecus aethiops* kidney epithelial cells¹ infected with West Nile Virus (WNV), TX 9410 (D0325) was gamma-irradiated on dry ice and sonicated to clarify supernatant.

Lot²: 70012727 Manufacturing Date: 22FEB2018

TEST	SPECIFICATIONS	RESULTS
Pre-Inactivation Identification by Infectivity in Vero cells ¹	Cell rounding and detachment	Cell rounding and detachment
Pre-Inactivation Sequencing of Species-Specific Region (~ 810 nucleotides)	Consistent with WNV	Consistent with WNV ³
Pre-Inactivation Titer by TCID ₅₀ Assay ^{4,5} in Vero cells ¹ by Cytopathic Effect	Report results	8.9 × 10 ⁸ TCID ₅₀ per mL
Pre-Inactivation Amplification of YFV Sequence by RT-PCR	~ 920 base pair amplicon	~ 920 base pair amplicon
Pre-Inactivation 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
Post-Inactivation Virus Inactivation		
Cell culture safety test for residual virus ⁶	No recovered virus	No recovered virus
NR-50806 was inoculated on Vero cells ¹ and evaluated for	No viable virus detected	No viable virus detected
cytopathic effect, viral antigen expression by indirect		
immunofluorescence assay ⁷ , and presence of viral RNA by		
real-time RT-PCR after serial passage8		

¹Vero; ATCC[®] CCL-81 ™

/Heather Couch/

Heather Couch 08 MAY 2019

Program Manager or designee, ATCC Federal Solutions

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

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

²Source of irradiated antigen: BEI Resources NR-49797 lot 70010130

³Sequence information for WNV, TX 9410 (D0325) is not available in the NCBI database; the nucleotide sequence obtained for NR-49797 lot 70010130 is highly similar to numerous WNV strains.

⁴Grown 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 7 days at 37°C with 5% CO₂

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.

⁶Performed at University of Texas Medical Branch, Galveston, Texas, USA

⁷Using Monoclonal Anti-Flavivirus Group Antigen, Clone D1-4G2-4-15 (BEI Resources NR-50327)

⁸The inactivated virus preparation was plated on Vero cells and incubated for 14 days at 37°C and 5% CO₂; cell lysate and supernatant from these cultures were passaged to fresh monolayers of Vero cells and incubated for 14 days at 37°C and 5% CO₂.