

Certificate of Analysis for NR-9538

West Nile Virus, Bird 114

Catalog No. NR-9538

Product Description: Cell lysate and supernatant from African green monkey (Vero) cells¹ infected with West Nile virus (WNV), Bird 114.

Lot²: 58276853 Manufacturing Date: 15SEP2008

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero Cells ¹	Report results	Cell rounding and sloughing
Identification by Indirect Fluorescent Antibody Assay³	Fluorescence observed	Fluorescence observed
Sequencing of West Nile Virus Specific Region	Consistent with WNV	Consistent with WNV
Titer by TCID ₅₀ Assay ^{4,5} in Vero Cells ¹	Report results	8.9 x 10 ⁷ TCID ₅₀ /mL
Functional Activity by RT-PCR Assay ⁶	~ 408 bp amplicon	~ 408 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 Test Article nucleic acid	None detected None detected	None detected None detected

¹Vero cells: ATCC® CCL-81™

Date: 30 DEC 2008 **Signature:** Signature on File

Title: Technical Manager, BEI Authentication or designee

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²NR-9538 was grown in Minimum Essential Medium containing Earle's salts and non-essential amino acids (Invitrogen™ 10370-021) supplemented with 2% irradiated fetal bovine serum (Cambrex® 14-471F), 2 mM L-glutamine (Invitrogen™ 25030-081), and 1 mM sodium pyruvate (Invitrogen™ 11360-070) for 5 days at 37°C and 5% CO₂.

³Using monoclonal antibody specific to the major envelope protein of WNV and Kunjin virus (Chemicon MAB8150)

⁴The 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.

⁵³ days at 37°C and 5% CO2

⁶WN233 and WN640c primers; Lanciotti, R. S., et al. "Rapid Detection of West Nile Virus from Human Clinical Specimens, Field-Collected Mosquitoes, and Avian Samples by a TaqMan Reverse Transcriptase-PCR Assay." <u>J. Clin. Microbiol.</u> 38 (2000): 4066-4071. PubMed: 11060069.

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