

Certificate of Analysis for NR-72

West Nile Virus, B 956

Catalog No. NR-72

(Derived from ATCC® VR-1510™)

Product Description: Cell lysate and supernatant from African green monkey (Vero) cells¹ infected with West Nile virus (WNV), B 956.

Lot²: 4594473 Manufacturing Date: 06MAR2006

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero Cells ¹	Report results	Cell rounding and sloughing
Identification by Indirect Fluorescent Antibody Assay ³	Fluorescence observed	Fluorescence observed
Titer by TCID ₅₀ Assay ^{4,5} in Vero Cells ¹	Report results	8.9 x 10 ⁷ TCID ₅₀ /mL
Sterility (21-day incubation) Harpo's HTYE broth ⁶ , 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: 29 July 2008 **Signature:** Signature on file

Title: Technical Manager, BEI Authentication or designee

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.

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²NR-72 was grown from ATCC[®] VR-1510[™] (Lot: 3399946) in Minimum Essential Medium containing Earle's salts and non-essential amino acids (Invitrogen[™] 10370-021) supplemented with 2% irradiated fetal bovine serum (Cambrex[®] 14-471E), 2 mM L-glutamine (Invitrogen[™] 25030-081), and 1 mM sodium pyruvate (Invitrogen[™] 11360-070) for 6 days at 37°C and 5% CO₂.

Using 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.

⁵10 days at 37°C and 5% CO₂

⁶Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.