

Certificate of Analysis for NR-49920

West Nile Virus, Goldblum

Catalog No. NR-49920

Product Description: West Nile virus (WNV), Goldblum was isolated from a human in Israel in 1953 or 1954. Each vial contains cell lysate and supernatant from *Cercopithecus aethiops* kidney epithelial cells¹ infected with WNV, Goldblum.

Passage History: SM23V1/V3 (Prior to deposit at BEI Resources/BEI Resources); SM = suckling mice: V = Vero cells¹

Lot^{2,3}: 64498507 Manufacturing Date: 13DEC2017

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero cells ¹	Cell rounding and detachment	Cell rounding and detachment
Sequencing of Species-Specific Region (~ 760 nucleotides)	Consistent with WNV	100% identity with WNV, twn9 (GenBank: HM051416) ⁴
Titer by TCID₅₀ Assay₅,6 in Vero cells¹ by Cytopathic Effect	Report results	2.8 x 10 ⁷ TCID ₅₀ per mL
Amplification of WNV Sequence by RT-PCR	~ 920 base pair amplicon	~ 920 base pair 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 ™

/Heather Couch/

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Program Manager or designee, ATCC Federal Solutions

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²The second viral passage at BEI Resources was performed by polyethylenimine (Polyplus-transfection® SA jetPEI® 101-10)-mediated transfection of extracted viral nucleic acid in order to remove contaminating mycoplasma.

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 6 days at 37°C with 5% CO₂

⁴There is no sequence information for WNV, Goldblum in the NCBI database or any of the various virology databases. The polyprotein gene sequence obtained for NR-49920 is identical to the published sequence of WNV, twn9, a WNV strain isolated from a human in Israel in 1953.

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.

⁶⁶ days at 37°C and 5% CO2

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