

West Nile Virus, Nea Santa

Catalog No. NR-49928

Product Description: West Nile virus (WNV), Nea Santa was isolated from a human in Greece in 2010. Vial contains cell lysate and supernatant from *Cercopithecus aethiops* kidney epithelial cells¹ infected with WNV, Nea Santa.

Passage History: XV1/V3 (Prior to deposit at BEI Resources/BEI Resources); X = Unknown; V = Vero cells¹

Lot^{2,3}: 70005269

Manufacturing Date: 07NOV2017

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero cells	Cell rounding and detachment	Cell rounding and sloughing
Sequencing of Species-Specific Region (740 nucleotides)	Consistent with WNV	Consistent with WNV ⁴
Titer by TCID ₅₀ Assay ^{5,6} in Vero cells ¹ by Cytopathic Effect	Report results	2.8 × 10 ⁸ TCID ₅₀ per mL
Amplification of WNV Sequence by RT-PCR	~ 890 base pair amplicon	~ 890 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 No growth No growth No growth No growth No growth	No growth No growth No growth No growth No growth 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™

²The second virus 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 4 days at 37°C with 5% CO₂

⁴Nucleotide sequence obtained for NR-49928 lot 70005269 is 100% identical to the closely related WNV strain 341/2010 (GenBank: KY594040.1; see Papa, A., et al. "West Nile Virus Lineage 2 from Blood Donor, Greece." *Emerg. Infect. Dis.* 18 (2012): 688-689. PubMed: 22469502) and WNV, strain Nea Santa-Greece-2010 (GenBank: HQ537483; see Papa, A., et al. "Genetic Characterization of West Nile Virus Lineage 2, Greece, 2010." *Emerg. Infect. Dis.* 17 (2011): 920-922. PubMed: 21529413) and consistent with 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.

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.

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