

West Nile Virus, R94224

Catalog No. NR-59933

This reagent is the property of the US Government

Product Description:

West Nile virus (WNV), R94224 was collected from the brain of a human on September 29, 2008, in Wisconsin, USA. NR-59933 was isolated on November 15, 2013. NR-59933 was produced by infecting *Chlorocebus aethiops* kidney epithelial cells (Vero; ATCC® CCL-81™) with the deposited material and incubating in Eagle's Minimum Essential Medium (ATCC® 30-2003™) supplemented with 2% fetal bovine serum (ATCC® 30-2020™) for 4 days at 37°C with 5% CO₂, passaging to fresh cells and incubating 4 days at 37°C with 5% CO₂ to produce this lot.

Passage History:

V(2)/V(2) (Arbovirus Reference Collection, Centers for Disease Control and Prevention/BEI Resources); V = Vero cells

Lot: 70071795

Manufacturing Date: 18JUN2024

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero Cells	Cell rounding and detachment	Cell rounding and detachment
Next-Generation Sequencing (NGS) of Complete Genome Using Illumina® MiSeq™ Platform	≥ 98% identity with WNV from USA (GenBank: AY646354) ¹	99.5% identity with WNV from USA (GenBank: AY646354) ¹
Titer by TCID ₅₀ Assay in Vero Cells by Cytopathic Effect ² (7 days at 37°C with 5% CO ₂)	Report results	5.0 × 10 ⁷ TCID ₅₀ /mL
Sterility (21-day incubation) Harpo's HTYE broth, 37°C and 26°C, aerobic ³ 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, aerobic	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

¹There is no sequence information for WNV, R94224 in the NCBI database or any of the various virology databases. The polyprotein gene sequence obtained for NR-59933 is very similar to the published sequence of WNV from USA (AY646354.1), a WNV strain isolated from a human in the USA in 2002.

²The 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.

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

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29 OCT 2024

Certificate of Analysis for NR-59933

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