

Certificate of Analysis for NR-49927

West Nile Virus, MX H 442

Catalog No. NR-49927

Product Description: West Nile virus (WNV), MX H 442 was isolated from a human in Sonora, Mexico in July 2004. Each vial contains cell lysate and supernatant from Cercopithecus aethiops kidney epithelial cells¹ infected with WNV, MX H 442.

Passage History: V1/V3 (Prior to deposit at BEI Resources/BEI Resources); V# = Vero cells¹

Lot^{2,3}: 70005267 Manufacturing Date: 31OCT2017

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero cells	Cell rounding and detachment	Cell rounding and detachment
Sequencing of Species-Specific Region (750 base pairs)	≥ 98% identity with WNV (GenBank: AY963774.1)	100% identity with WNV (GenBank: AY963774.1)
Titer by TCID ₅₀ Assay ^{4,5} in Vero cells¹ by Cytopathic Effect	Report results	8.9 × 10 ⁷ TCID ₅₀ per mL
Amplification of WNV Sequence by RT-PCR	~ 920 base pair amplicon	~ 920 base pair amplicon
Sterility (22-day incubation)		
Harpo's HTYE broth ⁶ , 37°C and 26°C, aerobic	No growth	No growth
Trypticase soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud broth, 37°C and 26°C, aerobic	No growth	No growth
Blood agar, 37°C, aerobic	No growth	No growth
Blood agar, 37°C, anaerobic	No growth	No growth
Thioglycollate broth, 37°C, anaerobic	No growth	No growth
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)	None detected	None detected
DNA detection by PCR of extracted Test Article nucleic acid	None detected	None detected

¹Vero: ATCC[®] CCL-81™

/Heather Couch/

Heather Couch 12 SEP 2018

Program Manager or designee, ATCC Federal Solutions

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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BEI Resources

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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 4 days at 37°C with 5% CO₂

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

⁵⁶ days at 37°C and 5% CO2

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