

Certificate of Analysis for NR-21719

Venezuelan Equine Encephalitis Virus, MX09-M50

Catalog No. NR-21719

Product Description: Cell lysate and supernatant from *Cercopithecus aethiops* kidney epithelial cells (Vero)¹ infected with Venezuelan equine encephalitis virus (VEEV), MX09-M50

Lot²: 61803610 Manufacturing Date: 07JUN2013

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity Using Vero Cells ¹	Report results	Refractile rounding and sloughing
Sequencing of Species-Specific Region (721 nucleotides)	Consistent with VEEV, MX09-M50	99% identity with VEEV, MX09-M50 (GenBank: KC344479)
Titer by TCID ₅₀ Assay ^{3,4} in Vero Cells ¹	Report results	2.8 × 10 ⁸ TCID ₅₀ per mL
Functional Activity by RT-PCR Assay	~ 750 bp amplicon	~ 750 bp amplicon
Sterility (21-day incubation) Harpo's HTYE broth ⁵ , 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 cells: ATCC[®] CCL-81™

Date: 10 NOV 2014 Signature: Mishael Q. Gypthe

Title: Technical Manager, BEI Authentication or designee

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²Grown in Dulbecco's Modified Eagle's Medium modified to contain 4 mM L-glutamine, 4500 mg/L glucose, 1 mM sodium pyruvate, and 1.5 g/L sodium bicarbonate (ATCC[®] 30-2002[™]) supplemented with 2% fetal bovine serum (ATCC[®] 30-2020[™]) for 2 days at 37°C with 5% CO₂.

³The 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.

⁴5 days at 37°C and 5% CO₂

⁵Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.