b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-41568

Madariaga Virus, BeAr436087 (formerly Eastern Equine Encephalitis Virus)

Catalog No. NR-41568

Product Description: Madariaga virus (MADV), BeAr436087 was isolated in Vero cells from a mosquito pool collected in Fortaleza, Brazil in 1985, and passaged twice in suckling mouse brains to generate RNA. An infectious cDNA clone of the viral genome was constructed and rescued by electroporation of *in vitro* transcribed RNA.

Passage History: SM2T1/V2 (Prior to deposit at BEI Resources/BEI Resources); SM = Suckling mice; T = Mammalian cell transfection; V = Vero 76 cells[1]

Lot[2]: 62111118 **Manufacturing Date: 02JUL2014**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero 76 cells[1]	Refractile cell rounding	Refractile cell rounding
Sequencing of Species-Specific Region (~ 910 nucleotides)	≥ 98% identity with MADV, BeAr436087 (GenBank: EF151503.1)	100% identity with MADV, BeAr436087 (GenBank: EF151503.1)
Titer by $TCID_{50}$ Assay[3,4] in Vero 76 cells[1] by Cytopathic Effect	Report results	1.6×10^9 $TCID_{50}$ per mL
Amplification of MADV Sequence by RT-PCR	~ 1100 base pair amplicon	~ 1100 base pair amplicon
Sterility (21-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

[1]*Cercopithecus aethiops* kidney epithelial cells (Vero 76; ATCC® CCL-1587™)
[2]Grown in Dulbecco's Modified Eagle's Medium containing 4 mM L-glutamine, 4500 mg per L glucose, 1 mM sodium pyruvate, and 1500 mg per L sodium bicarbonate (ATCC® 30-2002) supplemented with 2% fetal bovine serum (ATCC® 30-2020) for 2 days at 37°C with 5% CO_2
[3]The Tissue Culture Infectious Dose 50% ($TCID_{50}$) endpoint is the 50% infectious endpoint in cell culture. The $TCID_{50}$ 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% (LD_{50}) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the $TCID_{50}$ provides a measure of the titer (or infectivity) of a virus preparation.
[4]Assay plates were incubated 7 days at 37°C and 5% CO_2
[5]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

/Heather Couch/
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Program Manager or designee, ATCC Federal Solutions

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