

## **Certificate of Analysis for NR-21721**

## Venezuelan Equine Encephalitis Virus, 8138

Catalog No. NR-21721

**Product Description:** Cell lysate and supernatant from *Cercopithecus aethiops* kidney epithelial cells (Vero)<sup>1</sup> infected with Venezuelan equine encephalitis virus (VEEV), 8138

Lot<sup>2</sup>: 61803614 Manufacturing Date: 07JUN2013

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity Using Vero Cells <sup>1</sup>	Report results	Rounding and detachment
Sequencing of Species-Specific Region (616 nucleotides)	Consistent with VEEV, 8138	99% identity with VEEV, 8138 (GenBank: KC344471)
Titer by TCID <sub>50</sub> Assay <sup>3,4</sup> in Vero Cells <sup>1</sup>	Report results	1.6 × 10 <sup>8</sup> TCID <sub>50</sub> per mL
Functional Activity by RT-PCR Assay	~ 750 bp amplicon	~ 750 bp amplicon
Sterility (21-day incubation) Harpo's HTYE broth <sup>5</sup> , 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 <sub>2</sub>	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

<sup>&</sup>lt;sup>1</sup>Vero cells: ATCC<sup>®</sup> CCL-81™

Date: 02 APR 2014 Signature: Michael Q. Gyylu

Title: Technical Manager, BEI Authentication or designee

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<sup>&</sup>lt;sup>2</sup>Grown in Dulbecco's Modified Eagle's Medium (ATCC<sup>®</sup> 30-2002<sup>™</sup>) supplemented with 2% fetal bovine serum (ATCC<sup>®</sup> 30-2020<sup>™</sup>) for 2 days at 37°C with 5% CO<sub>2</sub>.

<sup>&</sup>lt;sup>3</sup>The Tissue Culture Infectious Dose 50% (TCID<sub>50</sub>) endpoint is the 50% infectious endpoint in cell culture. The TCID<sub>50</sub> 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<sub>50</sub>) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the TCID<sub>50</sub> provides a measure of the titer (or infectivity) of a virus preparation.

<sup>&</sup>lt;sup>4</sup>3 days at 37°C and 5% CO<sub>2</sub>

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