

## **Certificate of Analysis for NR-21717**

## Venezuelan Equine Encephalitis Virus, 309752

Catalog No. NR-21717

**Product Description:** Cell lysate and supernatant from *Cercopithecus aethiops* kidney epithelial cells (Vero)<sup>1</sup> infected with Venezuelan equine encephalitis virus (VEEV), 309752

Lot<sup>2</sup>: 61743291 Manufacturing Date: 23MAY2013

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity Using Vero Cells <sup>1</sup>	Report results	Rounding and detachment
Sequencing of Species-Specific Region (691 nucleotides)	Consistent with VEEV, 309752	99% identity with VEEV, 309752 (GenBank: KC344477)
Titer by TCID <sub>50</sub> Assay <sup>3,4</sup> in Vero Cells <sup>1</sup>	Report results	1.6 x 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: 20 AUG 2014 Signature: Michael Q. Gypthe

**Title:** Technical Manager, BEI Authentication or designee

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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<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>6</sup> days at 37°C and 5% CO<sub>2</sub>

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