b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-21725

Venezuelan Equine Encephalitis Virus, 65U206

Catalog No. NR-21725

Product Description: Cell lysate and supernatant from *Cercopithecus aethiops* kidney epithelial cells (Vero)[1] infected with Venezuelan equine encephalitis virus (VEEV), 65U206

Lot[2]: 62111121 **Manufacturing Date: 14NOV2013**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity Using Vero Cells[1]	Report results	Refractile rounding and sloughing
Sequencing of Species-Specific Region (685 nucleotides)	Consistent with VEEV, 65U206	99% identity with VEEV, 65U206 (GenBank: KC344446)
Titer by $TCID_{50}$ Assay[3,4] in Vero Cells[1]	Report results	1.6×10^8 $TCID_{50}$ 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	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
Sheep blood agar, 37°C, aerobic	No growth	No growth
Sheep 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]Vero cells: ATCC® CCL-81™

[2]Grown 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% 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]6 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.

Date: 09 DEC 2014 **Signature:** Michael Q. Gyrla

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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