b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-51177

Powassan Virus, 1427-62

Catalog No. NR-51177

Product Description:

Powassan virus (POWV), 1427-62 was isolated from an American red squirrel (*Tamiasciurus hudsonicus*) in August 1962 in Ontario, Canada.

Passage History:

SM3V1/V2 (Prior to deposit at BEI Resources/BEI Resources); SM = Suckling mouse; V = Vero cells[1]

Lot: 70021461[2] **Manufacturing Date: 12MAR2019**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero cells	Cell rounding and detachment	Cell rounding and detachment
Sequencing of Species-Specific Region (~ 2090 nucleotides)	≥ 98% identity with POWV, 1427-62 NS5 gene (GenBank: AF310942.1)	99.7% identity with POWV, 1427-62 NS5 gene (GenBank: AF310942.1)
Titer by $TCID_{50}$ Assay in Vero cells by Cytopathic Effect[1,3,4]	Report results	2.8×10^9 $TCID_{50}$ per mL
Amplification of POWV Sequence by RT-PCR	~ 1030 base pair amplicon	~ 1030 base pair amplicon
Sterility (21-day incubation)		
Harpo's HTYE broth, 37°C and 26°C, aerobic[5]	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; ATCC® CCL-81™)

[2] Grown in Eagle's Minimum Essential Medium containing Earle's Balanced Salt Solution, non-essential amino acids, 2 mM L-glutamine, 1 mM sodium pyruvate and 1.5 g/L of sodium bicarbonate (ATCC® 30-2003) supplemented with 2% fetal bovine serum (ATCC® 30-2020) for 7 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 13 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/
Heather Couch 27 SEP 2019
Program Manager or designee, ATCC Federal Solutions

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.

Support Provided by NIAID

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