

# **Certificate of Analysis for NR-51972**

#### Spondweni Virus, Chuku

#### Catalog No. NR-51972

#### **Product Description:**

Spondweni virus (ŚPOV), Chuku was isolated from the blood of a febrile human in Nigeria in 1952. NR-51972 lot 70032789 was produced by infecting *Aedes albopictus* cells (C6/36; ATCC® CRL-1660™) with the deposited material and incubating in Eagle's Minimum Essential Medium (ATCC® 30-2003™) supplemented with 2% fetal bovine serum (ATCC® 30-2020™) for 5 days at 28°C with 5% CO₂, then incubating the harvested material in Eagle's Minimum Essential Medium (ATCC® 30-2003™) supplemented with 2% fetal bovine serum (ATCC® 30-2020™) for an additional 5 days at 28°C with 5% CO₂.

### Passage History:

Unknown(X)/LLC-MK2(1)//C6/36(2) (Prior to deposit at Arbovirus Reference Collection (ARC)/ARC//BEI Resources); LLC-MK2 = *Macaca mulatta* kidney epithelial cells; C6/36 = *Aedes albopictus* cells

Lot: 70032789 Manufacturing Date: 28JUN2022

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in C6/36 Cells	Cell rounding and detachment	Cell rounding and detachment
Sequencing of Species-Specific Region (~ 870 nucleotides)	≥ 98% identity with SPOV, Chuku (GenBank: KX227369)	100% identity with SPOV, Chuku (GenBank: KX227369)
Titer by TCID <sub>50</sub> Assay in C6/36 Cells by Cytopathic Effect <sup>1</sup> (8 days at 28°C with 5% CO <sub>2</sub> )	Report results	2.8 × 10 <sup>7</sup> TCID <sub>50</sub> per mL
Amplification of SPOV Sequence by RT-PCR	~ 900 base pair amplicon	~ 900 base pair amplicon
Sterility (21-day incubation)		
Harpo's HTYE broth, 37°C and 26°C, aerobic <sup>2</sup>	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, aerobic	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

¹The Tissue Culture Infectious Dose 50% (TCID₅₀) endpoint is the 50% infectious endpoint in cell culture. The TCID₅₀ 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% (LD₅₀) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the TCID₅₀ provides a measure of the titer (or infectivity) of a virus preparation. ²Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

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