

Certificate of Analysis for NR-51647

Ross River Virus, Raratonga

Catalog No. NR-51647

Product Description:

Ross River virus (RRV), Raratonga was isolated from serum of a human subject in March 1980 in Raratonga, Cook Islands, New Zealand. NR-51647 lot 70028687 was produced by infecting *Aedes albopictus* mosquito larval epithelial clone C6/36 cells (C6/36; ATCC® CRL-1660™) and incubating in Dulbecco's Modified Eagle's Medium (ATCC® 30-2002) supplemented with 2% fetal bovine serum (ATCC® 30-2020) for 9 days at 28°C with 5% CO₂.

Passage History:

X(?)M(2)C(1)/C(3) (Prior to deposit at BEI Resources/BEI Resources); X = Unknown; M = Mosquito; C = C6/36 cells

Lot: 70028687 Manufacturing Date: 19DEC2019

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in C6/36 Cells	Cell rounding and detachment	Cell rounding and detachment
Sequencing of Species-Specific Region (~ 920 nucleotides)	≥ 98% identity with RRV	≥ 98% identity with RRV¹
Titer by TCID ₅₀ Assay in C6/36 Cells by Cytopathic Effect ² (9 days at 28°C with 5% CO ₂)	Report results	8.9 × 10 ⁹ TCID ₅₀ per mL
Amplification of RRV Sequence by RT-PCR	~ 940 base pair amplicon	~ 940 base pair amplicon
Sterility (21-day incubation)		
Harpo's HTYE broth, 37°C and 26°C, aerobic ³	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

¹Sequence information for RRV, Raratonga is not available in the NCBI database; nucleotide sequence obtained for NR-51647 lot 70028687 is ≥ 98% identical to numerous RRV strains.

/Heather Couch/

Heather Couch 03 AUG 2020

Program Manager or designee, ATCC Federal Solutions

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²The 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.
³Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.