

Certificate of Analysis for NR-51661

O'nyong-nyong Virus, UgMP 30

Catalog No. NR-51661

Product Description:

O'nyong-nyong virus (ONNV), UgMP 30 was isolated from human serum in 1959 in the Northern Province of Uganda. NR-51661 lot 70028681 was produced by infecting *Cercopithecus aethiops* kidney epithelial cells (Vero; ATCC[®] CCL-1586™) with the deposited material and incubating in Dulbecco's Modified Eagle's Medium (ATCC 30-2002™) supplemented with 2% fetal bovine serum (ATCC 30-2020™) for 2 days at 37°C with 5% CO₂.

Passage History:

X(9)V(1)/VE(2) (Prior to deposit to BEI Resources/BEI Resources); X = Unknown; V = Vero cells, VE = Vero E6 cells

Lot: 70028681 Manufacturing Date: 19SEP2019

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero E6 Cells	Cell rounding and detachment	Cell rounding and detachment
Sequencing of Species-Specific Region (~ 940 nucleotides)	≥ 98% identity with ONNV	≥ 98% identity with ONNV¹
Titer by TCID₅₀ Assay in Vero E6 Cells by Cytopathic Effect² (7 days at 37°C with 5% CO₂)	Report results	1.6 × 10 ⁷ TCID ₅₀ per mL
Amplification of ONNV Sequence by RT-PCR	~ 1040 base pair amplicon	~ 1040 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, 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

Sequence information for ONNV, UgMP 30 is not available in the NCBI database; nucleotide sequence obtained for NR-51661 lot 70028681 is 100% identical to ONNV, strain Gulu (GenBank: M20303.1) and ONNV, strain Ahero (GenBank: KX771232.1).

/Heather Couch/ Heather Couch

17 JUL 2020

Program Manager or designee, ATCC Federal Solutions

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BEI Resources www.beiresources.org E-mail: contact@beiresources.org Tel: 800-359-7370

Fax: 703-365-2898

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.