

Certificate of Analysis for NR-51664

Toscana Virus, ISS Phl 3

Catalog No. NR-51664

Product Description:

Toscana virus (TOSV), ISS PhI 3 was isolated from a sandfly (*Phlebotomus perniciosus*) in Monte Argentario, Toscana, Italy on July 17, 1971. NR-51664 lot 70028342 was produced by infecting *Cercopithecus aethiops* kidney epithelial cells (Vero E6; ATCC® CRL-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 9 days at 37°C with 5% CO₂.

Passage History:

P(?)SM(1)/VE(2) (Prior to deposit at BEI Resources/BEI Resources); P = Unknown; SM = Suckling mice; VE = Vero E6 cells

Lot: 70028342 Manufacturing Date: 26SEP2019

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero E6 Cells	Cell rounding and detachment	Cell rounding and detachment
Sequencing of Species-Specific Region (~ 950 nucleotides)	≥ 98% identity with TOSV, ISS PhI 3 (GenBank: X68414.1)	99.7% identity with TOSV, ISS Phl 3 (GenBank: X68414.1)
Titer by TCID ₅₀ Assay in Vero E6 Cells by Cytopathic Effect ¹ (4 days at 37°C with 5% CO ₂)	Report results	2.8 × 10 ⁶ TCID ₅₀ per mL
Amplification of TOSV Sequence by RT-PCR	~ 1010 base pair amplicon	~ 1010 base pair amplicon
Sterility (21-day incubation)		
Harpo's HTYE broth, 37°C and 26°C, aerobic ²	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. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

/Heather Couch/

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Program Manager or designee, ATCC Federal Solutions

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