

Certificate of Analysis for NR-9384

Seoul Virus, Houston Rat #4

Catalog No. NR-9384

Product Description:

Seoul virus (SEOV), Houston rat #4 deposited material was passaged three times in mycoplasma removal agent (MRA; MP Biomedicals™ 3050044) in order to remove contaminating mycoplasma. NR-9384 lot 70004097 was produced by infecting *Cercopithecus aethiops* kidney epithelial cells (Vero E6; ATCC® CRL-1586™) with the MRA-treated material and incubating in Eagle's Minimum Essential Medium (ATCC 30-2003™) supplemented with 2% fetal bovine serum (ATCC 30-2020™) for 14 days at 37°C with 5% CO₂.

Passage History:

X(?)/VE(7) (Prior to deposit at BEI Resources/BEI Resources); X = Unknown; VE = Vero E6 cells

Lot: 70004097 Manufacturing Date: 11DEC2018

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero E6 Cells	Cell rounding and detachment	Cell rounding and detachment
Sequencing of Species-Specific Region (~ 340 nucleotides)	≥ 98% identity with SEOV	≥ 98% identity with SEOV ¹
Titer by TCID ₅₀ Assay in Vero E6 Cells by Cytopathic Effect ² (14 days at 37°C with 5% CO ₂)	Report results	2.8 × 10 ⁵ TCID ₅₀ per mL
Amplification of SEOV Sequence by RT-PCR	~ 370 base pair amplicon	~ 370 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 SEOV, Houston rat #4 is not available in the NCBI database; nucleotide sequence obtained for NR-9384 lot 70004097 is ≥ 98% identical to numerous SEOV strains.

/Heather Couch/

Heather Couch 28 JUL 2020

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.

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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.