

Certificate of Analysis for NR-469

Junin Virus, Candid #1

Catalog No. NR-469

Product Description:

Junin virus (JUNV), Candid #1 is a vaccine strain that was developed in the late 1980s from JUNV, XJ, which was isolated from the first human infections with JUNV, the causative agent of Argentine Hemorrhagic Fever (AHF). The heavily attenuated vaccine strain, Candid #1, was developed through collaboration by United States Army Medical Research Institute of Infectious Diseases (USAMRIID), the Argentinian government and the US National Institutes of Health (NIH) by attenuation through serial passage in guinea pigs, mice and FRhK cells. NR-469 lot 70018115 was produced by infecting Cercopithecus aethiops kidney cells (Vero E6; ATCC® CCL-1586™) and incubating in Eagle's MEM (ATCC® 30-2003) supplemented with 2% fetal bovine serum (ATCC® 30-2020) for 14 days at 37°C with 5% CO₂. The first three virus passages at BEI Resources were performed with PlasmocinTM (InvivoGen ant-mpp) in order to remove contaminating mycoplasma, followed by three passages without PlasmocinTM to produce this lot.

Passage History:

X(?)/VE6(7) (Prior to deposit at BEI Resources/BEI Resources); X = Unknown; VE6 = Vero E6 cells

Lot: 70018115 Manufacturing Date: 27AUG2019

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero E6 Cells	Cell rounding and detachment	Cell rounding and detachment
Sequencing of Species-Specific Region (~ 820 nucleotides)	≥ 98% identity with JUNV, Candid #1 (GenBank: AY819707.2)	99.8% identity with JUNV, Candid #1 (GenBank: AY819707.2)
Titer by TCID ₅₀ Assay in Vero E6 Cells by qPCR ¹	Report results	1.6 × 10 ⁴ TCID ₅₀ per mL in 14 days at 37°C with 5% CO ₂
Amplification of JUNV Sequence by RT-PCR	~ 990 base pair amplicon	~ 990 base pair amplicon
Sterility (21-day incubation) Harpo's HTYE broth, 37°C and 26°C, aerobic² Trypticase Soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic Sheep blood agar, 37°C, aerobic Sheep blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic DMEM with 10% FBS, 37°C and 5% CO2	No growth	No growth
Mycoplasma Contamination Agar and broth culture (14-day incubation at 37°C) DNA detection by PCR of extracted Test Article nucleic acid	None detected None detected	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/

Heather Couch 13 FEB 2020

Program Manager or designee, ATCC Federal Solutions

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

Fax: 703-365-2898



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Tel: 800-359-7370 Fax: 703-365-2898