

Venezuelan Equine Encephalitis Virus, TC-83

Catalog No. NR-63

(Derived from ATCC® VR-1249™)

Product Description:

Venezuelan equine encephalitis virus (VEEV), TC-83 is an attenuated strain of VEEV that was derived from the original Trinidad donkey strain by serial passage in fetal guinea-pig heart cells. In-house sequencing confirms that the A3G reversion is absent, excluding this strain from select agent status. NR-63 was produced by infecting *Chlorocebus* (formerly *Cercopithecus*) *aethiops* kidney epithelial cells (Vero; ATCC® CCL-81™) with ATCC® VR-1249™ and incubating in Eagle's Minimum Essential Medium (ATCC® 30-2003™) supplemented with 2% fetal bovine serum (ATCC® 30-2020™) for 7 days at 37°C with 5% CO₂ to produce this lot.

Passage History:

Unknown/V(1) (Prior to deposit at BEI Resources/BEI Resources); V = Vero cells

Lot: 3579612

Manufacturing Date: 10MAR2004

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero Cells	Cell rounding and cell lysis	Cell rounding and cell lysis
Genotypic Analysis		
Sequencing within E2 Envelope Glycoprotein ¹	Consistent with VEE virus	Consistent with VEE virus
Confirmation of Absence of A3G Reversion by RACE-Sequencing	Absence of A3G reversion	Absence of A3G reversion (Table 1)
Titer by TCID₅₀ Assay in Vero Cells by Cytopathic Effect² (7 days at 37°C with 5% CO ₂)	Report results	1.6 × 10 ⁸ TCID ₅₀ /mL
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

¹Kinney, R. M., et al. "Attenuation of Venezuelan Equine Encephalitis Virus Strain TC-83 is Encoded by the 5'-Noncoding Region and the E2 Envelope Glycoprotein." *J. Virol.* 67 (1993): 1269–1277. PubMed: 7679745.

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. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Table 1: Confirmation of Absence of A3G Reversion by RACE-Sequencing

Sample	Total Reads	Sequence depth at nucleotide 3 (x coverage)	Total % of A	Mean Quality Score
NR-63 ¹	1.152 × 10 ⁶	134,815	99.99	>30

¹RACE (Rapid amplification of cDNA ends) PCR was performed to boost coverage at the third nucleotide. Amplicons were sequenced and aligned to Venezuelan equine encephalitis virus strain TC-83, complete genome (Genbank: MZ399798.1). The mean quality score of >30 suggests NR-63 does not contain an A3G reversion.

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Technical Manager or designee, ATCC Federal Solutions

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