

Certificate of Analysis for NR-56

Vaccinia Virus, Western Reserve (Mouse Adapted)

Catalog No. NR-56

(Derived from ATCC® VR-119™)

Product Description:

Vaccinia virus (VACV), Western Reserve (WR) (mouse adapted) was derived from the original New York City Board of Health (NYCBH) strain by intracerebral passages in mice. NR-56 lot 70055087 was produced by infecting *Cercopithecus aethiops* kidney cells (Vero; ATCC® CCL-81™) with seed material (BEI Resources lot 3580752) and incubating in Eagle's Minimum Essential Medium (ATCC® 30-2003™) supplemented with 2% fetal bovine serum (ATCC® 30-2020™) for 3 days at 37°C with 5% CO₂.

Passage History:

M(24), HeLa(Unk)/Vero(2) (Prior to deposit at BEI Resources/BEI Resources); M = Mouse brain; HeLa = *Homo sapiens* adenocarcinoma epithelial cells; Unk = unknown number of passages in HeLa, but included a plaque purification; Vero = *Cercopithecus aethiops* kidney cells

Lot: 70055087 Manufacturing Date: 16SEP2022

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero Cells	Cell rounding and detachment	Cell rounding and detachment
Sequencing of Species-Specific Region (~ 1010 nucleotides)	≥ 98% identity with VACV, WR (GenBank: AY243312.1)	99.7% identity with VACV, WR (GenBank: AY243312.1)
Titer by TCID ₅₀ Assay in Vero Cells by Cytopathic Effect ¹ (8 days at 37°C with 5% CO ₂)	Report results	2.8 × 10 ⁶ TCID ₅₀ per mL
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. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

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