SUPPORTING INFECTIOUS DISEASE RESEARCH

Dengue Virus Type 3 (DEN-3), Philippines/H87/1956

Catalog No. NR-80

Derived from ATCC[®] VR-1256 (NIAID V-576-001-022)

Product Description:

Dengue virus type 3 (DEN-3), Philippines/H87/1956 was derived from ATCC[®] VR-1256 (V-576-001-022). ATCC[®] VR-1256 was derived from ATCC[®] VR-216, which was isolated from human serum in the Philippines in 1956. NR-80 lot 70061552 was produced by infecting *Chlorocebus* (formerly *Cercopithecus*) *aethiops* kidney epithelial cells (Vero; ATCC[®] CCL-81[™]) 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.

Passage History:

SM(21)/V(5) (Prior to deposit at BEI Resources/BEI Resources); SM = Suckling mice; V = Vero cells

Lot: 70061552

Manufacturing Date: 14JUL2023

TEST	SPECIFICATIONS	RESULTS
Identification by Fluorescent Antibody Assay in Vero Cells	Fluorescence observed	Fluorescence observed
Sequencing of Species-Specific Region (~ 890 nucleotides)	≥ 98% identity with DEN-3, Philippines/H87/1956 (GenBank: M93130)	99.6% identity with DEN-3, Philippines/H87/1956 (GenBank: M93130)
Titer by TCID ₅₀ Assay in Vero Cells by Cytopathic Effect and Fluorescent Antibody Test ^{1,2} (12 days at 37°C with 5% CO ₂)	Report results	1.6 × 10 ⁷ 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

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. ²Using mouse anti-dengue 3 (Millipore 8703)

³Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

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