

Dengue Virus Type 4, H241 (Tissue Culture Adapted)

Catalog No. NR-86

(Derived from ATCC® VR-1490™)

Product Description:

Dengue virus type 4 (DEN-4), H241 was deposited at ATCC® by Dr. W. Brandt and was used to prepare ATCC® VR-217™ in suckling mouse. VR-1490™ was derived through tissue culture adaptation of ATCC® VR-217™. NR-86 lot 70078289 was produced by infecting *Macaca mulatta* kidney epithelial cells (LLC-MK2 derivative cells; ATCC® CCL-7.1™) with NR-86 lot 70037388 and incubating in Eagle's Minimum Essential Medium containing Earle's Balanced Salt Solution, non-essential amino acids, 2 mM L-glutamine, 1 mM sodium pyruvate and 1.5 g per L of sodium bicarbonate (ATCC® 30-2003™) supplemented with 2% fetal bovine serum (ATCC® 30-2020™) for 8 days at 37°C with 5% CO₂.

Passage History:

SM(24),LLC-MK2(5)/LLC-MK2(4) (Prior to deposit at BEI Resources/BEI Resources); SM = Suckling mice

Lot: 70037388

Manufacturing Date: 29JUL2020

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in LLC-MK2 Cells	Cell rounding and sloughing	Cell rounding and sloughing
Identification by Indirect Fluorescent Antibody (IFA) Assay ¹	Fluorescence observed	Fluorescence observed
Sequencing of Species-Specific Region (~ 480 nucleotides)	≥ 98% sequence identity with DEN-4, H241 (GenBank: AY947539.1)	99.2% sequence identity with DEN-4, H241 GenBank: AY947539.1)
Titer by TCID ₅₀ Assay in LLC-MK2 Cells by Immunofluorescent Antibody ^{1,2} (9 days at 37°C with 5% CO ₂)	Report results	1.6 × 10 ⁶ TCID ₅₀ /mL
Amplification of DEN-4, H241 Sequence by RT-PCR	~ 500 base pair amplicon	~ 500 base pair amplicon
Sterility (21-day incubation) Harpo's HTYE broth, 37°C and 26°C, aerobic ³ 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, aerobic	No growth No growth No growth No growth No growth No growth No growth	No growth No growth No growth No growth No growth 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

¹Immunofluorescent stain performed using mouse anti-dengue complex (Millipore cat. no. MAB8705) and anti-mouse IgG FITC (Millipore cat. no. AP124F)

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.

/Sonia Bjorum Brower/

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Technical Manager or designee, ATCC Federal Solutions

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