b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-86

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% CO_2.

Passage History:

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

Lot: 70078289 Manufacturing Date: 14SEP2022[1]

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in LLC-MK2 Cells	Cell rounding and detachment	Cell rounding and detachment
Identification by Indirect Fluorescent Antibody (IFA) Assay[2]	Fluorescence observed	Fluorescence observed
Sequencing of Species-Specific Region Polyprotein gene (~ 460 nucleotides)	≥ 98% sequence identity with DEN-4, H241 polyprotein gene (GenBank: KR011349.2)	99.6% sequence identity with DEN-4, H241 polyprotein gene (GenBank: KR011349.2)
Titer by $TCID_{50}$ Assay in LLC-MK2 Cells by Immunofluorescent Antibody[3] (9 days at 37°C with 5% CO_2)	Report results	2.8×10^4 $TCID_{50}$/mL
Sterility test (BacT/ALERT 3D)		
iAST bottle (aerobic) at 32.5°C, 14-day incubation	No growth	No growth
iNST bottle (anaerobic) at 32.5°C, 14-day incubation	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

[1]Dispensed at BEI Resources 10OCT2025

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

[3]The Tissue Culture Infectious Dose 50% ($TCID_{50}$) endpoint is the 50% infectious endpoint in cell culture. The $TCID_{50}$ 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% (LD_{50}) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the $TCID_{50}$ provides a measure of the titer (or infectivity) of a virus preparation.

/Sonia Bjorum Brower/
Sonia Bjorum Brower 21 JAN 2026
Technical Manager or designee, ATCC Federal Solutions

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

Support Provided by NIAID

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