

Dengue Virus Type 4, H241 (Tissue Culture Adapted)

Catalog No. NR-86

(Derived from ATCC® VR-1490™)

Product Description: Cell lysate and supernatant from *Macaca mulatta* kidney epithelial cells¹ infected with dengue virus type 4 (DEN-4), H241

Lot²: 63069010

Manufacturing Date: 14NOV2014

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity Using LLC-MK2 Derivative Cells ¹	Report results	Cell rounding and degeneration
Identification by Indirect Fluorescent Antibody (IFA) Assay ³	Fluorescence observed	Fluorescence observed
Sequencing of Species-Specific Region (490 nucleotides)	Consistent with DEN-4, H241	99% identity with DEN-4, H241 (GenBank: AY947539)
Titer by TCID ₅₀ Assay ^{4,5} in LLC-MK2 Derivative Cells ¹ with IFA Readout ³	Report results	1.6 × 10 ⁵ TCID ₅₀ per mL
RT-PCR Assay of Extracted RNA Using DEN Specific Primers ⁶	~ 500 bp amplicon	~ 500 bp amplicon
Sterility (21-day incubation) Harpo's HTYE broth ⁷ , 37°C and 26°C, aerobic 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 and 5% CO ₂	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 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

¹LLC-MK2 derivative cells (ATCC® CCL-7.1™)

²Grown 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/L of sodium bicarbonate (ATCC® 30-2003) supplemented with 2% fetal bovine serum (ATCC® 30-2020) for 7 days at 37°C with 5% CO₂.

³Using Anti-Dengue Virus Complex Antibody, clone D3-2H2-9-21 (Millipore MAB8705)

⁴The 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.

⁵7 days at 37°C and 5% CO₂

⁶Lanciotti, R. S., et al. "Rapid Detection and Typing of Dengue Viruses from Clinical Samples by Using Reverse Transcriptase-Polymerase Chain Reaction." *J. Clin. Microbiol.* 30 (1992): 545-551. PubMed: 1372617.

⁷Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Date: 05 FEB 2015

Signature: 

Title: Technical Manager, BEI Authentication or designee

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