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: Cell lysate and supernatant from *Macaca mulatta* kidney epithelial cells[1] infected with dengue virus type 4 (DEN-4), H241

Lot[2]: 64347313 **Manufacturing Date: 05JUL2016**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity Using LLC-MK2 Derivative Cells[1]	Report results	Cell rounding and detachment
Identification by Indirect Fluorescent Antibody (IFA) Assay[3]	Fluorescence observed	Fluorescence observed
Sequencing of Species-Specific Region (491 nucleotides)	Consistent with DEN-4, H241	99% identity with DEN-4, H241 (GenBank: KR011349)
Titer by $TCID_{50}$ Assay[4,5] in LLC-MK2 Derivative Cells[1] with IFA Readout[3]	Report results	2.32×10^5 $TCID_{50}$ 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	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 and 5% CO_2	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]LLC-MK2 derivative cells (ATCC® CCL-7.1™)

[2]Grown 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% CO_2.

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

[4]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.

[5]8 days at 37°C and 5% CO_2

[6]Lanciotti, 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.

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

Date: 16 NOV 2016 **Signature:**

BEI Resources Authentication

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