b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-48801

Dengue Virus Type 4, 703-4

Catalog No. NR-48801

Product Description:

Dengue virus type 4 (DEN-4), 703-4 was isolated from a human in Thailand in 1994. NR-48801 was produced by infecting *Aedes albopictus* mosquito larval epithelial cells (clone C6/36; ATCC® CRL-1660™) with BEI Resources seed lot 62819333 and incubating in Eagle's Minimum Essential Medium (ATCC® 30-2003™) supplemented with 2% fetal bovine serum (ATCC® 30-2020™) for 7 days at 28°C in an aerobic atmosphere with 5% CO_2 to produce this lot.

Passage History:

C6/36(X)/C6/36(3) (Prior to deposit at BEI Resources/BEI Resources); C6/36 = *Aedes albopictus* mosquito larval epithelial cells

Lot: 70070788 **Manufacturing Date: 28AUG2024**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in C6/36 Cells	Inconsistent; cell enlargement and detachment	Inconsistent; cell enlargement and detachment
Identification by Indirect Fluorescent Antibody (IFA) Assay[1]	Fluorescence observed	Fluorescence observed
Sequencing of Species-Specific Region (~ 910 nucleotides)	≥ 98% identity with DEN-4, 703-4 (GenBank: AF231726)	99.6% identity with DEN-4, 703-4 (GenBank: AF231726)
Titer by $TCID_{50}$ Assay in C6/36 Cells by Immunofluorescent Assay[1,2] (7 days at 28°C with 5% CO_2)	Report results	2.8×10^7 $TCID_{50}$/mL
Amplification of DENV-4 Sequence by RT-PCR	~ 1000 base pair amplicon	~ 1000 base pair amplicon
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

[1]Using Anti-Dengue Virus Complex Antibody (Millipore MAB8705)

[2]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.

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

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Certificate of Analysis for NR-48801

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.

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