b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-49749

Dengue Virus Type 2, DKA 8521

Catalog No. NR-49749

Product Description: Cell lysate and supernatant from *Aedes albopictus* mosquito larval clone C6/36 cells[1] infected with dengue virus type 2 (DEN-2), DKA 8521

Passage History: C2/C2 (Prior to deposit at BEI Resources/BEI Resources); C# = Number of passages in C6/36 cells[2]

Lot[3]: 63777133 **Manufacturing Date: 20JAN2016**

TEST	SPECIFICATIONS	RESULTS
Identification by Indirect Fluorescent Antibody (IFA) Assay[4]	Fluorescence observed	Fluorescence observed
Sequencing of Species-Specific Region (511 nucleotides)	Consistent with DEN-2	Consistent with DEN-2[5]
Titer by $TCID_{50}$ Assay[6,7] in C6/36 Cells[1] with IFA Readout[8]	Report results	8.9×10^5 $TCID_{50}$ per mL
Amplification of Dengue Virus Sequence by RT-PCR	~ 500 bp amplicon	~ 500 bp amplicon
Sterility (21-day incubation)		
Harpo's HTYE broth[9], 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]*Aedes albopictus* clone C6/36 cells (ATCC® CRL-1660™)

[2]The first virus passage at BEI Resources was performed by lipofectamine transfection of extracted viral nucleic acid in order to remove contaminating mycoplasma.

[3]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 28°C with 5% CO_2.

[4]Using Anti-Dengue Virus Type II Antibody (Millipore MAB8702)

[5]Sequence information for DEN-2, DKA 8521 is not available in the NCBI database; nucleotide sequence obtained for NR-49749, Lot No. 63777133 is highly similar to several DEN-2 strains isolated in Singapore in 2009 and 2010.

[6]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.

[7]7 days at 28°C and 5% CO_2

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

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

Date: 19 MAY 2016 **Signature:** Michael R. Gyrla

BEI Resources Authentication

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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