b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-49713

Dengue Virus Type 1, UIS 998

Catalog No. NR-49713

Product Description: Clarified supernatant from *Aedes albopictus* mosquito larval clone C6/36 cells[1] infected with dengue virus type 1 (DEN-1), UIS 998

Passage History: C2/C3 (Prior to deposit at BEI Resources/BEI Resources); C# = Number of passages in C6/36 cells

Lot[2]: 99 **Manufacturing Date: 25OCT2016**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity Using C6/36 Cells[1]	Report results	Syncytia formation
Identification by Indirect Fluorescent Antibody (IFA) Assay[3]	Fluorescence observed	Fluorescence observed
Sequencing of Species-Specific Region (884 nucleotides)	Consistent with DEN-1	Consistent with DEN-1[4]
Titer by $TCID_{50}$ Assay[5,6] in C6/36 Cells[1] with IFA Readout[7]	Report results	1.6×10^7 $TCID_{50}$ per mL
Amplification of Dengue Virus Sequence by RT-PCR	~ 1000 bp amplicon	~ 1000 bp amplicon
Sterility (21-day incubation)		
Harpo's HTYE broth[8], 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]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.

[3]Using Anti-Dengue Virus Type I Antibody (Millipore MAB 8701) and Anti-Dengue Virus Complex Antibody (Millipore MAB 8705)

[4]Sequence information for DEN-1, UIS 998 is not available in the NCBI database; nucleotide sequence obtained for NR-49713, Lot No. 99 is ~ 99% identical to DENV-1/CO/BID-V3390/2007 (GenBank: GQ868569), a DEN-1 strain that was also isolated in Santander, Colombia in 2007.

[5]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.

[6]8 days at 28°C and 5% CO_2

[7]Using Anti-Dengue Virus Type I Antibody (Millipore MAB 8701)

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

Date: 24 JUL 2017 **Signature:**

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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BEI Resources
www.beiresources.org

E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898