b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-3785

Dengue Virus Type 1, 12150

Catalog No. NR-3785

Product Description: Cell lysate and supernatant from *Aedes albopictus* clone C6/36 cells[1] infected with dengue virus type 1 (DEN-1), 12150.

Lot[2,3]: 58613972 **Manufacturing Date: 03NOV2009**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in C6/36 Cells[1]	Report results	Cell rounding and syncytia
Identification by Indirect Fluorescent Antibody (IFA) Assay[4]	Fluorescence observed	Fluorescence observed
Sequencing of DEN-1 Specific Sequence (~ 888 nucleotides)	Consistent with DEN-1	Consistent with DEN-1
Titer by $TCID_{50}$ Assay in C6/36 Cells with IFA Readout[1,5,6]	Report results	1.6 x 10^7 $TCID_{50}$/mL
Functional Activity by RT-PCR Assay Using DEN-1 Specific Primers	~ 1200 bp amplicon	~ 1200 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 Test Article nucleic acid	None detected	None detected

[1]*Aedes albopictus* clone C6/36 cells (ATCC® CRL-1660™)

[2]The deposited virus preparation was determined by PCR to be contaminated with *Mycoplasma*. Genomic RNA was extracted from the deposited material and transfected into *Aedes albopictus* clone C6/36 cells. The resulting virus preparation was shown to be free of mycoplasma contamination and was used as the source virus for this lot.

[3]Grown in Minimum Essential Medium containing Earle's salts and non-essential amino acids (Invitrogen™ 10370-021) supplemented with 2% fetal bovine serum (ATCC® 30-2020™), 2 mM L-glutamine (Invitrogen™ 25030-081), and 1 mM sodium pyruvate (Invitrogen™ 11360-070) for 8 days at 28°C with 5% CO_2

[4]Using monoclonal antibody specific to dengue complex (Chemicon MAB8705)

[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]12 days at 28°C with 5% CO_2

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

Date: 29 MAR 2010 **Signature:** Signature on File

Title: Technical Manager, BEI Authentication or designee

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.

ATCC® is a trademark of the American Type Culture Collection.
You are authorized to use this product for research use only. It is not intended for human use.

Support Provided by NIAID

Biodefense and Emerging Infections Research Resources Repository
P.O. Box 4137
Manassas, VA 20108-4137 USA
www.beiresources.org

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

© 2010 American Type Culture Collection (ATCC). All rights reserved.