b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-3792

Dengue Virus Type 2, BC27/96

Catalog No. NR-3792

Product Description: Cell lysate and supernatant from *Aedes albopictus* clone C6/36 cells[1] infected with dengue virus type 2 (DEN-2), BC27/96.

Lot[2,3]: 58613978 **Manufacturing Date: 26FEB2010**

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-2 Specific Region (856 nucleotides)	Consistent with DEN-2	Consistent with DEN-2
Titer by $TCID_{50}$ Assay in C6/36 Cells with IFA Readout[1,5,6]	Report results	1.6×10^9 $TCID_{50}$/mL
Functional Activity by RT-PCR Assay Using DEN-2 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 7 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: 23 JUN 2010 **Signature:** Signature on File

Title: Technical Manager, BEI Authentication or designee

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