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SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-49750

Dengue Virus Type 2, PR 06-65-361

Catalog No. NR-49750

Product Description: Cell lysate and supernatant from *Aedes albopictus* mosquito larval clone C6/36 cells[1] infected with dengue virus type 2 (DEN-2), PR 06-65-361

Passage History: C1/C2 (Prior to deposit at BEI Resources/BEI Resources); C# = Number of passages in C6/36 cells

Lot[2]: 64004713 **Manufacturing Date: 06JUN2016**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in C6/36 cells	Cell rounding and detachment	Cell rounding and detachment
Sequencing of Species-Specific Region (885 nucleotides)	Consistent with DEN-2	Consistent with DEN-2[3]
Titer by $TCID_{50}$ Assay[4,5] in C6/36 Cells[1] with IFA Readout[6]	Report results	2.8×10^6 $TCID_{50}$ per mL
Amplification of Dengue Virus Sequence by RT-PCR	~ 1000 bp amplicon	~ 1000 bp amplicon
Sterility (21-day incubation)		
Harpo's HTYE broth[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 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 10 days at 28°C with 5% CO_2.

[3]Sequence information for DEN-2, PR 06-65-361 is not available in the NCBI database; nucleotide sequence obtained for NR-49750, Lot No. 64004713 is highly similar to numerous contemporaneous DEN-2 strains.

[4]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.

[5]10 days at 28°C and 5% CO_2

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

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

Date: 31 OCT 2016 **Signature:**

BEI Resources Authentication

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