b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-49754

Dengue Virus Type 3, S 9311 (PRS 228762)

Catalog No. NR-49754

Product Description:

Dengue virus type 3 (DENV3), S 9311 (PRS 228762) was isolated from a human in 1963 in Puerto Rico.

Passage History:

MXC1/C2 (Prior to deposit at BEI Resources/BEI Resources); M = Mosquito; X = Unknown; C = C6/36 cells[1]

Lot: 70016042[2] **Manufacturing Date: 05JUL2019**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in C6/36 cells[1]	Report results	Cell rounding and detachment
Identification by Indirect Fluorescent Antibody (IFA) Assay[3]	Fluorescence observed	Fluorescence observed
Sequencing of Species-Specific Region (~ 880 nucleotides)	≥ 98% identity with DENV3, DENV3/Puerto-Rico/1963/ PRS-228762-AC27 (GenBank: KT452800.1)	100% identity with DENV3, DENV3/Puerto-Rico/1963/ PRS-228762-AC27 (GenBank: KT452800.1)
Titer by $TCID_{50}$ Assay in in C6/36 Cells with IFA Readout[1,4,5,6]	Report results	1.6×10^6 $TCID_{50}$ per mL
Amplification of DEN-3 Sequence by RT-PCR	~ 1000 base pair amplicon	~ 1000 base pair amplicon
Sterility (21-day incubation)		
Harpo's HTYE broth, 37°C and 26°C, aerobic[7]	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
Blood agar, 37°C, aerobic	No growth	No growth
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 3 Antibody (Millipore MAB8703)

[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] Assay plates were incubated 14 days at 28°C and 5% CO_2

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

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

/Heather Couch/
Heather Couch 25 OCT 2019
Program Manager or designee, ATCC Federal Solutions

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