

Dengue Virus Type 2, 1349

Catalog No. NR-12219

Product Description: Cell lysate and supernatant from *Aedes albopictus* clone C6/36 cells¹ infected with dengue virus type 2 (DEN-2), 1349.

Lot²: 58737211

Manufacturing Date: 20AUG2009

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in C6/36 Cells ¹	Report results	Syncytia and fusion
Identification by Indirect Fluorescent Antibody (IFA) Assay ³	Fluorescence observed	Fluorescence observed
Sequencing of DEN-2 Specific Sequence (666 nucleotides)	Consistent with DEN-2	Consistent with DEN-2
Titer by TCID ₅₀ Assay in C6/36 Cells with IFA Readout ^{1,4,5}	Report results	1.6 x 10 ⁸ 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 ⁶ , 37°C and 26°C, aerobic Trypticase soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic Sheep blood agar, 37°C, aerobic Sheep blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic DMEM with 10% FBS, 37°C and 5% CO ₂	No growth No growth No growth No growth No growth No growth No growth	No growth No growth No growth No growth No growth No growth No growth
Mycoplasma Contamination Agar and broth culture (14-day incubation at 37°C) DNA Detection by PCR of Test Article nucleic acid	None detected None detected	None detected None detected

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

²DEN-2, 1349 was deposited by Dr. Rebeca Rico-Hesse of the Department of Virology and Immunology, Southwest Foundation for Biomedical Research, San Antonio, Texas. NR-12219 was grown from deposited virus seed in Minimum Essential Medium containing Earle's salts and non-essential amino acids (Invitrogen™ 10370-021) supplemented with 2% irradiated fetal bovine serum (Cambrex® 14-471F), 2 mM L-glutamine (Invitrogen™ 25030-081), and 1 mM sodium pyruvate (Invitrogen™ 11360-070) for 7 days at 28°C with 5% CO₂

³Using monoclonal antibody specific to DEN-2 (Chemicon MAB8705)

⁴The Tissue Culture Infectious Dose 50% (TCID₅₀) endpoint is the 50% infectious endpoint in cell culture. The TCID₅₀ 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% (LD₅₀) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the TCID₅₀ provides a measure of the titer (or infectivity) of a virus preparation.

⁵8 days at 28°C with 5% CO₂

⁶Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Date 20 NOV 2009

Signature: Signature on File

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