

Dengue Virus Type 2, 429557

Catalog No. NR-12216

Product Description: Cell lysate and supernatant from *Aedes albopictus* clone C6/36 cells¹ infected with dengue virus type 2 (DEN-2), 429557.

Lot²: 58526832

Manufacturing Date: 20MAY2009

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in C6/36 Cells ¹	Report results	Rounding and syncytia
Identification by Indirect Fluorescent Antibody (IFA) Assay ³	Fluorescence observed	Fluorescence observed
Sequencing of DEN-2 Specific Sequence (562 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	8.9 x 10 ⁷ TCID ₅₀ /mL
Functional Activity by RT-PCR Assay Using DEN-2 Specific Primers	~ 1200 bp amplicon	~ 1200 bp amplicon
Bacterial Sterility (BacT/ALERT[®] 3D Microbial Detection System) 14-day incubation of NR-12216: i NST culture bottle, 32°C, anaerobic i AST culture bottle, 32°C, aerobic	No growth No growth	No growth No growth
Fungal Sterility (21-day incubation) Harpo's HTYE broth ⁶ , 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic Sheep blood agar, 37°C and 26°C, aerobic	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, 429557 was deposited by Dr. Rebeca Rico-Hesse of the Department of Virology and Immunology, Southwest Foundation for Biomedical Research, San Antonio, Texas. NR-12216 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 MAB8702)

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.

⁵7 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: 28 FEB 2011

Signature:

Dorothy C. Young

Title:

Technical Manager, BEI Authentication or designee

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