

Dengue Virus Type 3 (DEN-3), Philippines/H87/1956

Catalog No. NR-80

Derived from ATCC® VR-1256 (V-576-001-022)

Product Description: Cell lysate and supernatant from African green monkey kidney (Vero) cells¹ infected with DEN-3, Philippines/H87/1956.

Lot²: 64286309

Manufacturing Date: 27MAY2016

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero Cells ¹	Report results	Cell rounding and sloughing
Identification by Indirect Fluorescent Antibody (IFA) Assay ³	Fluorescence observed	Fluorescence observed
Sequencing of Species-Specific Region (839 nucleotides)	Consistent with DEN-3, Philippines/H87/1956	99% identity with DEN-3, Philippines/H87/1956 (GenBank: AB609590)
Titer by TCID ₅₀ Assay ^{4,5} in Vero Cells ¹ with IFA Readout ⁶	Report results	8.9 x 10 ⁴ 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 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 extracted Test Article nucleic acid	None detected None detected	None detected None detected

¹Vero cells: ATCC® CCL-81™

²Grown 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 9 days at 37°C with 5% CO₂.

³Using Anti-Dengue Virus Type 3 Antibody (Millipore MAB8703)

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.

⁵9 days at 37°C and 5% CO₂

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

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

Date: 09 SEP 2016

Signature:



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