

**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) cells<sup>1</sup> infected with DEN-3, Philippines/H87/1956.

**Lot<sup>2</sup>: 70010661**

**Manufacturing Date: 12DEC2017**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero Cells <sup>1</sup>	Report results	Cell rounding and sloughing
Identification by Indirect Fluorescent Antibody (IFA) Assay <sup>3</sup>	Fluorescence observed	Fluorescence observed
Titer by TCID <sub>50</sub> Assay <sup>4,5</sup> in Vero Cells <sup>1</sup> with IFA Readout <sup>3</sup>	Report results	8.9 × 10 <sup>7</sup> TCID <sub>50</sub> per mL
Amplification of Dengue Virus Sequence by RT-PCR	~ 1000 bp amplicon	~ 1000 bp amplicon
Sequencing of Species-Specific Region (888 nucleotides)	Consistent with DEN-3, Philippines/H87/1956	99% identity with DEN-3, Philippines/H87/1956 (GenBank: M93130)
<b>Sterility (21-day incubation)</b> Harpo's HTYE broth <sup>6</sup> , 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 <sub>2</sub>	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 No growth No growth
<b>Mycoplasma Contamination</b> 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

<sup>1</sup>Vero cells: ATCC® CCL-81™

<sup>2</sup>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 12 days at 37°C with 5% CO<sub>2</sub>.

<sup>3</sup>Using Anti-Dengue Virus Complex Antibody (Millipore MAB8705)

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

<sup>5</sup>12 days at 37°C and 5% CO<sub>2</sub>

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

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Program Manager or designee, ATCC Federal Solutions

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