

## **Certificate of Analysis for NR-84**

## Dengue Virus Type 2 (DEN-2), New Guinea C (NGC)

Catalog No. NR-84

(Derived from ATCC® VR-1584™)

Product Description: DEN-2, NGC was isolated in 1944 from the serum of a febrile man in New

Guinea.

Lot<sup>1</sup>: 70019561 Manufacturing Date: 28NOV2018

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in LLC-MK2 Derivative Cells <sup>2</sup>	Cell rounding and detachment	Cell rounding and detachment
Identification by Indirect Fluorescent Antibody (IFA) Assay³	Fluorescence observed	Fluorescence observed
Sequencing of Species-Specific Region (810 nucleotides)	≥ 99% identity with DEN-2, NGC (GenBank: KM204118)	99.8% identity with DEN-2, NGC (GenBank: KM204118)
Titer by TCID <sub>50</sub> Assay <sup>4,5</sup> in LLC-MK2 Derivative Cells <sup>2</sup> by IFA <sup>3</sup>	Report results	$1.6 \times 10^6  TCID_{50}  per  mL$
Amplification of DEN-2 Sequence by RT-PCR	~ 970 base pair amplicon	~ 970 base pair amplicon
Sterility (21-day incubation) 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 Blood agar, 37°C, aerobic 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
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

<sup>&</sup>lt;sup>1</sup>Lot 70019561 of NR-84 was produced by infecting LLC-MK2 derivative cells with BEI Resources NR-84 lot 62905413 and incubating 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 8 days at 37°C with 5% CO<sub>2</sub>.

/Heather Couch/ Heather Couch

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Program Manager or designee, ATCC Federal Solutions

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<sup>&</sup>lt;sup>2</sup>Macaca mulatta kidney epithelial cells (LLC-MK2 derivative cells; ATCC® CCL-7.1™)

<sup>&</sup>lt;sup>3</sup>Virus presence detected using Anti-Dengue Virus Type II, clone 3H5-1 Antibody (Millipore MAB8702).

<sup>&</sup>lt;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>Assay plates were incubated 7 days at 37°C and 5% CO<sub>2</sub>.

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