b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

# Certificate of Analysis for NR-82

## Dengue Virus Type 1 (DEN-1), Hawaii

## Catalog No. NR-82

Derived from ATCC® VR-1254™

## Product Description:

Dengue virus type 1 (DEN-1), Hawaii was isolated from the pooled serum of six patients in Hawaii in 1944. DEN-1, Hawaii was deposited to ATCC® in 1964 by Dr. Charles L. Wisseman, Jr. and used to prepare ATCC® VR-71™ in suckling mouse. ATCC® VR-71™ was transferred to the National Institute of Allergy and Infectious Diseases (NIAID). NIAID V-574-001-522 was derived from ATCC® VR-71™ in mouse brain. NIAID V-574-001-522 was transferred to ATCC® in 1997 and used to produce ATCC® VR-1254™ in mouse brain, which was used as the inoculum for NR-82. NR-82 lot 70065873 was produced by infecting *Chlorocebus* (formerly *Cercopithecus) aethiops* kidney epithelial cells (Vero; ATCC® CCL-81™) and incubating in Eagle's Minimum Essential Medium (ATCC® 30-2003™) supplemented with 2% fetal bovine serum (ATCC® 30-2020™) for 13 days at 33°C with 5% $CO_2$.

## Passage History:

Mouse(124),SM(2)/Vero(6) (Prior to deposit at BEI Resources/BEI Resources); SM = Suckling mouse; V = Vero cells

**Lot: 70065873** **Manufacturing Date: 07SEP2022[1]**

| TEST | SPECIFICATIONS | RESULTS |
|---|---|---|
| **Identification by Infectivity in Vero Cells** | Cell rounding and detachment | Cell rounding and detachment |
| **Sequencing of Species-Specific Region** (~ 860 nucleotides) | ≥ 98% identity with DEN-1, Hawaii (GenBank: KM204119) | 99.3% identity with DEN-1, Hawaii (GenBank: KM204119.1) |
| **Titer by $TCID_{50}$ Assay in Vero Cells by Immunofluorescent Antibody[2,3]** (14 days at 33°C with 5% $CO_2$) | Report results | $5.00 \times 10^7$ $TCID_{50}$/mL |
| **Amplification of DENV Sequence by RT-PCR** | ~ 1000 base pair amplicon | ~ 1000 base pair amplicon |
| **Sterility (21-day incubation)** | | |
| Harpo's HTYE broth, 37°C and 26°C, aerobic[4] | No growth | No growth |
| Trypticase Soy broth, 37°C and 26°C, aerobic | No growth | No growth |
| Sabouraud broth, 37°C and 26°C, aerobic | No growth | No growth |
| Sheep blood agar, 37°C, aerobic | No growth | No growth |
| Sheep blood agar, 37°C, anaerobic | No growth | No growth |
| Thioglycollate broth, 37°C, anaerobic | No growth | No growth |
| DMEM with 10% FBS, 37°C, aerobic | No growth | No growth |
| **Mycoplasma Contamination** | | |
| Agar and broth culture (14-day incubation at 37°C) | None detected | None detected |
| DNA detection by PCR of extracted Test Article nucleic acid | None detected | None detected |

[1]Bulk thawed and dispensed 22JAN2024

[2]The Tissue Culture Infectious Dose 50% ($TCID_{50}$) endpoint is the 50% infectious endpoint in cell culture. The $TCID_{50}$ 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% ($LD_{50}$) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the $TCID_{50}$ provides a measure of the titer (or infectivity) of a virus preparation.

[3]Confirmed using mouse anti-Dengue complex primary antibody (Millipore MAB8705) and goat anti-mouse secondary antibody (Millipore AP124f).

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

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