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SUPPORTING INFECTIOUS DISEASE RESEARCH

# Certificate of Analysis for NR-43282

## Dengue Virus Type 3, DENV-3/US/BID-V1043/2006

## Catalog No. NR-43282

This reagent is the property of the U.S. Government.

**Product Description:** Cell lysate and supernatant from *Aedes albopictus* mosquito larval clone C6/36 cells[1] infected with dengue virus type 3, DENV-3/US/BID-V1043/2006

**Lot[2]: 70011363** **Manufacturing Date: 06DEC2016**

| TEST | SPECIFICATIONS | RESULTS |
|---|---|---|
| **Identification by Indirect Fluorescent Antibody (IFA) Assay[3]** | Fluorescence observed | Fluorescence observed |
| **Sequencing of Species-Specific Region (890 nucleotides)** | Consistent with DENV-3/US/BID-V1043/2006 | 99% identity with DENV-3/US/BID-V1043/2006 (GenBank: EU482555) |
| **Titer by $TCID_{50}$ Assay[4,5] in C6/36 Cells[1] with IFA Readout[3]** | Report results | $2.8 \times 10^5$ $TCID_{50}$ per mL |
| **Functional Activity by RT-PCR Assay** | ~ 1 Kb amplicon | ~ 1 Kb amplicon |
| **Sterility (21-day incubation)** | | |
| Harpo's HTYE broth[6], 37°C and 26°C, aerobic | 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 and 5% $CO_2$ | 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] *Aedes albopictus* clone C6/36 cells (ATCC® CRL-1660™)
[2] 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 7 days at 28°C with 5% $CO_2$.
[3] Using Anti-Dengue Virus Complex Antibody (Millipore MAB8705)
[4] 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.
[5] 7 days at 28°C and 5% $CO_2$
[6] Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

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