

## **Certificate of Analysis for NR-50533**

## Genomic RNA from Dengue Virus Type 4, UIS 497

## Catalog No. NR-50533

## **Product Description:**

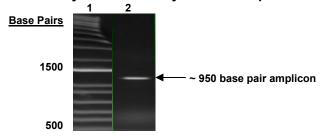
Genomic RNA was isolated from a preparation of cell lysate and supernatant from Aedes albopictus mosquito larval epithelial cells (clone C6/36; ATCC<sup>®</sup> CRL-1660™) infected with dengue virus type 4 (DEN-4), UIS 497 (BEI Resources lot 70026347) using QIAamp® Viral RNA Mini Kit (Qiagen® 52906). The viral genomic RNA is in a background of cellular nucleic acid and carrier RNA.

Lot: 70048383 Manufacturing Date: 27OCT2021

TEST	SPECIFICATIONS	RESULTS
Genotypic Analysis Sequencing of species-specific region	Consistent with DEN-4	≥ 99.9% identity with DENV-4/ CO/BID-V3411/2004 (GenBank: GQ868584) <sup>1</sup>
Functional Activity by RT-PCR Amplification <sup>2</sup>	~ 950 base pair amplicon	~ 950 base pair amplicon (Figure 1)
Concentration Using Qubit™ Quantitation System³ (Post vial)	Report results	10.1 ng per µL
Virus Inactivation  10% of total yield inoculated on C6/36 cells and evaluated for cytopathic effect and by immunofluorescent antibody test after serial passage <sup>4</sup>	No viable virus detected	No viable virus detected

Sequence information for DEN-4, UIS 497 is not available in the NCBI database; nucleotide sequence obtained for NR-50533 lot 70048383 is > 99%

Figure 1: Functional Activity of NR-50533 by RT-PCR Amplification



Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder Lane 2: PCR product from 1 µL of NR-50533

/Heather Couch/

**Heather Couch** 15 FEB 2022

Program Manager or designee, ATCC Federal Solutions

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identical to DENV-4/CO/BID-V3411/2004 (GenBank: GQ868584), a DEN-4 strain that was also isolated in Santander, Colombia in 2004. 
<sup>2</sup>Reverse transcription was performed using an iScript™ cDNA Synthesis Kit (Bio-Rad 170-8891) with 10 µL of NR-50533 in a 20 µL reaction; PCR was performed using iTaq™ DNA Polymerase (Bio-Rad 170-8870) with 5 μL of cDNA in a 50 μL reaction. <sup>3</sup>Using Qubit™ RNA High Sensitivity Assay (Thermo Fisher Q32855)

<sup>&</sup>lt;sup>4</sup>Use of the QIAamp<sup>®</sup> Viral RNA Mini Kit has been demonstrated to consistently inactivate dengue viruses as shown by the absence of cytopathic effect and viral antigen expression by indirect immunofluorescence after plating the entire extract on virus-susceptible cells.