b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

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 clone C6/36 cells[1] infected with dengue virus type 4 (DEN-4), UIS 497.

Lot[2]: 370 **Manufacturing Date: 10MAR2017**

TEST	SPECIFICATIONS	RESULTS
Sequencing of Species-Specific Region (826 nucleotides)	Consistent with DEN-4	Consistent with DEN-4[3]
Functional Activity by RT-PCR Amplification[4]	~ 1100 bp amplicon	~ 1100 bp amplicon (Figure 1)
Total RNA Content by RiboGreen® Measurement (Viral, Cellular, and Carrier)	Report results	1.4 ng per 100 µL
Viral RNA Content by Droplet Digital RT-PCR	Report results	6.7×10^3 copies per µL
Virus Inactivation 10% of total yield inoculated on C6/36 cells[1] and evaluated for expression of viral antigen[1,5]	No viable virus detected	No viable virus detected

[1]*Aedes albopictus* clone C6/36 cells (ATCC® CRL-1660™)

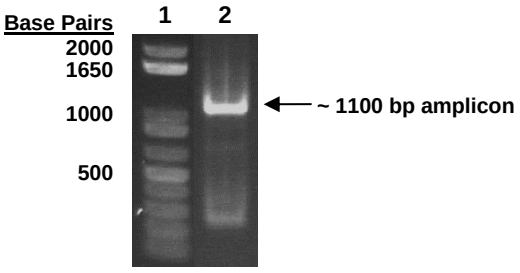
[2]Nucleic acid was extracted from a preparation of DEN-4 (BEI Resources NR-49724, Lot 63721385) using a QIAamp® Viral RNA Mini Kit (Qiagen 52906).

[3]Sequence information for DEN-4, UIS 497 is not available in the NCBI database; nucleotide sequence obtained for NR-50533, Lot No. 370 is > 99% identical to DENV-4/CO/BID-V3410/2004 (GenBank: GQ868583) and DENV-4/CO/BID-V3411/2004 (GenBank: GQ868584), two DEN-4 strains that were also isolated in Santander, Colombia in 2004.

[4]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.

[5]Use of the QIAamp® 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.

Figure 1



Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder
Lane 2: NR-50533

Date: 29 NOV 2017 **Signature:**

BEI Resources Authentication

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

Support Provided by NIAID

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