

## **Certificate of Analysis for NR-2771**

## Genomic RNA from Dengue Virus Type 3 (DEN-3), Philippines/H87/1956

Catalog No. NR-2771

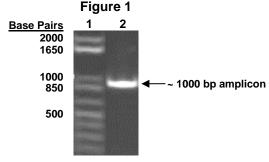
**Product Description:** Genomic RNA was isolated from a preparation of cell lysate and supernatant from African green monkey (Vero) cells<sup>1</sup> infected with DEN-3, Philippines/H87/1956.

Lot<sup>2</sup>: 64522915 Manufacturing Date: 26SEP2016

TEST	SPECIFICATIONS	RESULTS
Sequencing of Species-Specific Region (897 nucleotides)	Consistent with DEN-3 Philippines/H87/1956 Consistent with NR-80	99% identity with DEN-3 Philippines/H87/1956 (GenBank: AB609590) Consistent with NR-80
Functional Activity by RT-PCR Amplification <sup>3</sup>	~ 1000 bp amplicon	~ 1000 bp amplicon (see Figure 1)
Total RNA Content by RiboGreen® Measurement (Viral, Cellular, and Carrier)	Report results	287 ng per 100 μL
Viral RNA Content by Droplet Digital RT-PCR	Report results	1.1 × 10 <sup>6</sup> copies per μL
Virus Inactivation 10% of total yield inoculated on Vero cells¹ and evaluated for immunofluorescence 4,5	No virus detected	No virus detected

<sup>&</sup>lt;sup>1</sup>Vero cells: ATCC<sup>®</sup> CCL-81™

<sup>&</sup>lt;sup>5</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.



Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder

Lane 2: NR-2771

Date: 23 NOV 2016 Signate

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.

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<sup>&</sup>lt;sup>2</sup>Nucleic acid was extracted from a preparation of DEN-3, Philippines/H87/1956 (BEI Resources NR-80, Lot 64286309) using a QIAamp® Viral RNA Mini Kit (Qiagen 52906).

³Reverse transcription was performed using an iScript™ cDNA Synthesis Kit (Bio-Rad 170-8891) with 10 µL of NR-2771 in a 20 µL reaction; PCR was performed using iTaq™ DNA Polymerase (Bio-Rad 170-8870) with 2 µL of cDNA in a 25 µL reaction.

<sup>&</sup>lt;sup>4</sup>Using Anti-Dengue Virus Complex Antibody, clone D3-2H2-9-21 (Millipore MAB8705)