

Certificate of Analysis for NR-4287

Genomic RNA from Dengue Virus Type 1, Hawaii

Catalog No. NR-4287

Product Description:

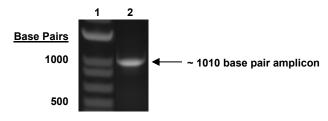
Genomic RNA was isolated from a preparation of cell lysate and supernatant from *Cercopithecus aethiops* kidney epithelial cells (Vero; ATCC® CCL-81™) infected with dengue virus type 1 (DEN-1), Hawaii [BEI Resources NR-82 (derived from ATCC® VR-1254™) lot 70018416] using QIAamp® Viral RNA Mini Kit (Qiagen® 52904). The viral genomic RNA is in a background of cellular nucleic acid and carrier RNA.

Lot: 70036101 Manufacturing Date: 24SEP2020

TEST	SPECIFICATIONS	RESULTS
Genotypic Analysis Sequencing of species-specific region (~ 400 nucleotides)	≥ 98% identity with DEN-1, Hawaii (GenBank: EU848545.1)	99.5% identity with DEN-1, Hawaii (GenBank: EU848545.1)
Functional Activity by RT-PCR Amplification ¹	~ 1010 base pair amplicon	~ 1010 base pair amplicon (Figure 1)
Estimated Concentration (post-dilution) by RiboGreen® Measurement (Viral, Cellular and Carrier)²	Report results	5.3 ng per 100 μL (0.05 μg per mL)
Estimated Amount per Vial ²	Report Results	5.3 ng
Virus Inactivation 10% of total yield inoculated on Vero cells and evaluated for cytopathic effect and immunofluorescence ^{3,4}	No viable virus detected	No viable virus detected

¹Amplified using iTaq™ Universal SYBR Green One-step Kit (Bio-Rad® 172-5151) with 5 μL of NR-4287 in a 50 μL reaction

Figure 1: Functional Activity of NR-4287 by RT-PCR Amplification



Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder Lane 2: PCR product from 1 μL of NR-4287

/Heather Couch/

Heather Couch 30 MAR 2021

Program Manager or designee, ATCC Federal Solutions

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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BEI Resources www.beiresources.org E-mail: contact@beiresources.org
Tel: 800-359-7370

Fax: 703-365-2898

²Measurement is determined pre-vial prior to dilution due to the limit of detection of the quantification method

³Using Anti-Dengue Virus Type I Antibody, clone 15F3-1 (Millipore MAB8701)

⁴Use 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.