

## **Certificate of Analysis for NR-50610**

## Genomic RNA from Zika Virus, R116265 (Human/2016/Mexico)

## Catalog No. NR-50610

## **Product Description:**

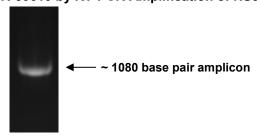
Genomic RNA was extracted from a preparation of cell lysate and supernatant from *Cercopithecus aethiops* kidney epithelial cells infected with Zika virus (ZIKV), R116265 (Human/2016/Mexico).<sup>1</sup>

Lot: 70020575<sup>2</sup> Manufacturing Date: 20NOV2018

TEST	SPECIFICATIONS	RESULTS
Genotypic Analysis Sequencing of species-specific region (950 nucleotides)	≥ 98% identity with Zika virus	100% identity with ZIKV, R116265 (Human/2016/Mexico) complete genome (GenBank: KX766029.1)
Functional Activity by RT-PCR Amplification <sup>3</sup> N3-N4 gene	~ 1080 base pair amplicon	~ 1080 base pair amplicon (Figure 1)
Pre-Vial Concentration by RiboGreen® Measurement (Viral, Cellular and Carrier) <sup>4</sup>	Report results	4.3 ng per 100 μL (0.04 μg/mL)
Estimated Amount per Vial <sup>4</sup>	Report results	4.3 ng
Genome Copy Number by ddPCR (Post-Vial) <sup>5</sup>	Report results	1.1 x 10 <sup>4</sup> genome copies/mL
Virus Inactivation 10% of total yield inoculated on Vero cells and evaluated for cytopathic effect after serial passage <sup>1,6</sup>	No viable virus detected	No viable virus detected

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

Figure 1: Functional Activity of NR-50610 by RT-PCR Amplification of NS3-NS4



PCR product from 5 µL of NR-50610

/Heather Couch/

Heather Couch 13 SEP 2019

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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<sup>&</sup>lt;sup>2</sup>Nucleic acid was extracted from a preparation of ZIKV, R116265 (BEI Resources NR-50551 lot 70001080), using a QIAamp<sup>®</sup> Viral RNA Mini Kit (Qiagen 52906).

³Amplified using iTaq™ Universal SYBR Green One-step Kit (Bio-Rad 172-5151) with 5 µL of NR-50610 in a 50 µL reaction

<sup>&</sup>lt;sup>4</sup>Measurement is determined pre-vial prior to dilution due to the limit of detection of the quantification method

<sup>&</sup>lt;sup>5</sup>ddPCR data was obtained post-vial from 9 replicates on the BioRad QX200 Droplet Digital PCR (ddPCR™) System

<sup>&</sup>lt;sup>6</sup>Use of the QIAamp<sup>®</sup> Viral RNA Mini Kit has been demonstrated to consistently inactivate flaviviruses as shown by the absence of cytopathic effect (CPE) and viral RNA expression by RT-PCR after plating the entire extract on virus-susceptible cells for two passages.