

## **Certificate of Analysis for NR-51473**

## Genomic RNA from Zika Virus, DAK AR 41524

## Catalog No. NR-51473

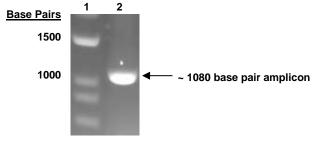
**Product Description:** Genomic RNA was isolated from a preparation of cell lysate and supernatant from *Cercopithecus aethiops* kidney epithelial cells<sup>1</sup> infected with Zika virus (ZIKV), DAK AR 41524.

Lot<sup>2</sup>: 70021061 Manufacturing Date: 18DEC2018

| TEST  | SPECIFICATIONS   | RESULTS   |
|---|--|---|
| Genotypic Analysis  |  |   |
| Sequencing of species-specific region (930 nucleotides)   | ≥ 98% identity with ZIKV, DAK AR<br>41524 (GenBank: KX601166.2)<br>Consistent with source organism<br>(BEI Resources NR-50338) | 99.9% identity with ZIKV, DAK AR<br>41524 (GenBank: KX601166.2)<br>Consistent with NR-50338 |
| Functional Activity by RT-PCR Amplification <sup>3</sup>  |  |   |
| NS3-NS4 polyprotein gene  | ~ 1080 base pair amplicon  | ~ 1080 base pair amplicon<br>(Figure 1)   |
| Pre-Vial Concentration by RiboGreen® Measurement (Viral, Cellular and Carrier) <sup>4</sup>               | Report results   | 24.8 ng per 100 μL (0.248 μg/mL)  |
| Estimated Amount per Vial <sup>4</sup>  | Report results   | 24.8 ng   |
| Genome Copy Number by ddPCR (Post-Vial) <sup>5</sup>  | Report results   | 2.4 × 10 <sup>3</sup> genome copies/mL  |
| Virus Inactivation  |  |   |
| 10% of total yield inoculated on Vero cells <sup>1</sup> and evaluated for cytopathic effect <sup>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-51473 by RT-PCR Amplification of NS3-NS4 polyprotein gene



Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder Lane 2: PCR product from 0.5 µL of NR-51473

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<sup>&</sup>lt;sup>2</sup>Nucleic acid was extracted from a preparation of ZIKV, DAK AR 41524 (BEI Resources NR-50338 lot 64237873), using a QIAamp<sup>®</sup> Viral RNA Mini Kit (Qiagen 52906).

<sup>&</sup>lt;sup>3</sup>Reverse transcription was performed using an iScript™ cDNA Synthesis Kit (Bio-Rad 170-8891) with 10 μL of NR-51473 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>&</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 100% of ZIKV as shown by the absence of cytopathic effect (CPE) after plating the entire extract on virus-susceptible cells.



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Program Manager or designee, ATCC Federal Solutions

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