

Certificate of Analysis for NR-51223

Genomic RNA from Zika Virus, BeH819015, Recombinant Infectious Clone

Catalog No. NR-51223

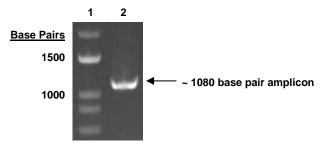
Product Description: Genomic RNA was isolated from a preparation of cell lysate and supernatant from Cercopithecus aethiops kidney epithelial cells1 infected with Zika virus (ZIKV), BeH819015 recombinant infectious clone.

Lot²: 70020822 Manufacturing Date: 10JAN2019

TEST	SPECIFICATIONS	RESULTS
Genotypic Analysis		
Sequencing of species-specific region (~ 840 nucleotides)	≥ 98% identity with ZIKV, BeH819015 (GenBank: KU365778) Consistent with source organism (BEI Resources NR-51129)	100% identity with ZIKV, BeH819015 (GenBank: KU365778) Consistent with NR-51129
Functional Activity by RT-PCR Amplification ³		
NS3-NS4 polyprotein gene	~ 1080 base pair amplicon	~ 1080 base pair amplicon (Figure 1)
Pre-Vial Concentration by RiboGreen® Measurement (Viral, Cellular and Carrier) ⁴	Report results	3.2 ng per 100 µL (0.032 µg/mL)
Estimated Amount per Vial ⁴	Report results	3.2 ng
Genome Copy Number by ddPCR (Post-Vial) ⁵	Report results	1.8 × 10 ⁴ genome copies/µL
Virus Inactivation		
10% of total yield inoculated on Vero cells ¹ and evaluated for cytopathic effect ⁶	No viable virus detected	No viable virus detected

¹Vero cells, clone E6: ATCC[®] CRL-1586™

Figure 1: Functional Activity of NR-51223 by RT-PCR Amplification of NS3-NS4 Polyprotein Gene



Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder Lane 2: PCR product from 0.2 µL of NR-51223

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²Nucleic acid was extracted from a preparation of ZIKV, BeH819015 (BEI Resources NR-51129 lot 70011621), 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-51223 in a 20 µL reaction; PCR was performed using iTaq™ DNA Polymerase (Bio-Rad 170-8870) with 2 µL of cDNA in a 50 µL reaction.

⁴Measurement is determined pre-vial, prior to dilution, due to the limit of detection of the quantification method.

⁵ddPCR data was obtained post-vial from 9 replicates on the BioRad QX200 Droplet Digital PCR (ddPCR™) System.

⁶Use of the QIAamp[®] 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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/Heather Couch/ Heather Couch

13 JUN 2019

Program Manager or designee, ATCC Federal Solutions

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