

## **Certificate of Analysis for NR-50240**

## Zika Virus, PRVABC59

Catalog No. NR-50240

**Product Description:** Cell lysate and supernatant from *Cercopithecus aethiops* kidney epithelial cells<sup>1</sup> infected with Zika virus (ZIKV), PRVABC59

Passage History: V3/V2 (Centers for Disease Control/BEI Resources); V# = Number of passages

in Vero cells

Lot<sup>2</sup>: 64112564 Manufacturing Date: 15MAR2016

TEST	SPECIFICATIONS	RESULTS
Infectivity in Vero E6 Cells <sup>1</sup>	Report results	Cell rounding and detachment
Sequencing of Species-Specific Region (1716 nucleotides)	Consistent with ZIKV, PRVABC59	99% identity with ZIKV, PRVABC59 (GenBank: KU501215) <sup>3</sup>
Titer by TCID <sub>50</sub> Assay in Vero E6 Cells <sup>1,4,5</sup>	Report results	1.6 × 10 <sup>8</sup> TCID <sub>50</sub> per mL
Viral Genome Copy Number by Droplet Digital RT-PCR <sup>6</sup>	Report results	~8 × 10 <sup>6</sup> copies per µL
Sterility (21-day incubation) Harpo's HTYE broth <sup>7</sup> , 37°C and 26°C, aerobic Trypticase soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic Sheep blood agar, 37°C, aerobic Sheep blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic DMEM with 10% FBS, 37°C and 5% CO <sub>2</sub>	No growth	No growth
Mycoplasma Contamination  Agar and broth culture (14-day incubation at 37°C)  DNA Detection by PCR of Test Article nucleic acid	None detected None detected	None detected None detected

<sup>&</sup>lt;sup>1</sup>Vero 76, clone E6: ATCC<sup>®</sup> CRL-1586™

**Date:** 15 MAR 2017

Signature:

**BEI Resources Authentication** 

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<sup>&</sup>lt;sup>2</sup>Grown in Eagle's Minimum Essential Medium containing Earle's Balanced Salt Solution, non-essential amino acids, 2 mM L-glutamine, 1 mM sodium pyruvate (ATCC<sup>®</sup> 30-2003) and 2% fetal bovine serum (ATCC<sup>®</sup> 30-2020) for 5 days at 37°C with 5% CO<sub>2</sub>

<sup>&</sup>lt;sup>3</sup>The complete coding sequence of NR-50240, Lot No. 64112564 has also been determined (GenBank: KX087101).

<sup>&</sup>lt;sup>4</sup>The Tissue Culture Infectious Dose 50% (TCID<sub>50</sub>) is the 50% infectious endpoint in cell culture. The TCID<sub>50</sub> is the dilution of virus that under the conditions of the assay can be expected to infect 50% of the culture vessels inoculated, just as a Lethal Dose 50% (LD<sub>50</sub>) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the TCID<sub>50</sub> provides a measure of the titer (or infectivity) of a virus preparation.

<sup>&</sup>lt;sup>6</sup>Estimated from viral RNA content of NR-50244, Lot No. 64112564, without accounting for efficiency of nucleic acid extraction; presented for information only; users should verify copy number in their own assays before using either NR-50240 or NR-50244 as quantitative standards.
<sup>7</sup>Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.