

## Certificate of Analysis for NR-50066

## Zika Virus, IbH 30656

Catalog No. NR-50066

**Product Description:** Cell lysate and supernatant from *Cercopithecus aethiops* kidney epithelial cells (Vero)<sup>1</sup> infected with Zika virus (ZIKV), IbH 30656

**Passage History:** SM21V1/V3 (Prior to deposit at BEI/BEI); SM# = Number of passages in suckling mice; V# = Number of passages in Vero cells

Lot<sup>2</sup>: 64066122 Manufacturing Date: 21FEB2016

TEST	SPECIFICATIONS	RESULTS
Infectivity in Vero Cells <sup>1</sup>	Report results	Cell rounding and detachment
Sequencing of Species-Specific Region (915 nucleotides)	Consistent with ZIKV, IbH 30656	100% identity with ZIKV, IbH 30656 (GenBank: HQ234500) <sup>3</sup>
Titer by TCID <sub>50</sub> Assay in Vero Cells <sup>1,4,5</sup>	Report results	1.6 × 10 <sup>8</sup> TCID <sub>50</sub> per mL
Sterility (21-day incubation) Harpo's HTYE broth <sup>6</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 cells: ATCC<sup>®</sup> CCL-81™

Date: 06 APR 2016 Signature

**BEI Resources Authentication** 

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>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-50066, Lot No. 63856751 has also been determined (GenBank: KU963574).

<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>5</sup>8 days at 37°C with 5% CO₂

<sup>&</sup>lt;sup>6</sup>Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.