

## **Certificate of Analysis for NR-50183**

## Zika Virus, FLR

Catalog No. NR-50183

Product Description: Cell lysate and supernatant from Aedes albopictus mosquito larval clone C6/36 cells<sup>1</sup> infected with Zika virus (ZIKV), FLR

Passage History: C1/C2 (Baylor College of Medicine/BEI Resources); C# = Number of passages in C6/36 cells

Lot<sup>2</sup>: 64103036 Manufacturing Date: 14MAR2016

TEST	SPECIFICATIONS	RESULTS
Infectivity in C6/36 Cells <sup>1</sup>	Report results	Cell rounding and detachment
Sequencing of Species-Specific Region (1710 nucleotides)	Consistent with ZIKV, FLR	99% identity with ZIKV, FLR (GenBank: KU820897) <sup>3</sup>
Titer by TCID <sub>50</sub> Assay in C6/36 Cells <sup>1,4,5</sup> with IFA Readout <sup>6</sup>	Report results	$8.9 \times 10^7 \text{ TCID}_{50} \text{ per mL}$
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>Aedes albopictus clone C6/36 cells: ATCC<sup>®</sup> CRL-1660™

**Date:** 19 APR 2016

**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 28°C with 5% CO<sub>2</sub>

<sup>3</sup>The complete coding sequence of NR-50183, Lot No. 64103036 has also been determined (GenBank: KX087102).

<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>7 days at 28°C with 5% CO<sub>2</sub>

<sup>&</sup>lt;sup>6</sup>Using mouse monoclonal antibody to Zika virus NS1 protein (Aalto Bio Reagents catalog no. AZ 1225, clone 2701106)

<sup>&</sup>lt;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.