

## Certificate of Analysis for NR-50547

## Zika Virus, PRVABC59, Infected Cell Lysate, Gamma-Irradiated

Catalog No. NR-50547

**Product Description:** A crude preparation of Vero E6<sup>1</sup> cells infected with Zika virus (ZIKV), PRVABC59 was gamma-irradiated (5 x 10<sup>6</sup> RADs) on dry ice.

Lot<sup>2,3</sup>: 70003287 Manufacturing Date: 02MAR2017

TEST	SPECIFICATIONS	RESULTS
Pre-Inactivation Titer by TCID <sub>50</sub> Assay in Vero E6 cells <sup>4,5</sup>	Report results	$2.8 \times 10^7 \text{ TCID}_{50} \text{ per mL}$
Pre-Inactivation Sterility (21-day incubation)		
Harpo's HTYE broth <sup>6</sup> , 37°C and 26°C, aerobic	No growth	No growth
Trypticase soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud broth, 37°C and 26°C, aerobic	No growth	No growth
Blood agar, 37°C, aerobic	No growth	No growth
Blood agar, 37°C, anaerobic	No growth	No growth
Thioglycollate broth, 37°C, anaerobic	No growth	No growth
DMEM with 10% FBS, 37°C and 5% CO <sub>2</sub>	No growth	No growth
Pre-Inactivation Mycoplasma Contamination		
Agar and broth culture (14-day incubation at 37°C)	None detected	None detected
DNA detection by PCR of extracted Test Article nucleic	None detected	None detected
Viral Genome Copy Number		
Droplet Digital RT-PCR <sup>7</sup>	Report results	1.1 x 10 <sup>7</sup> genome copies per µL
Quantitative Real-Time RT-PCR	Report results	3.0 x 10 <sup>7</sup> genome copies per µL
Amplification of Zika Virus NS3/NS4 Coding Region by RT-PCR	~ 1100 base pair amplicon	~ 1100 base pair amplicon
Virus Inactivation		
Cell culture safety test for residual virus <sup>8</sup>	No recovered virus	No recovered virus
NR-50547 was inoculated on Vero E6 cells and evaluated	No viable virus detected	No viable virus detected
for cytopathic effect and viral antigen expression by		
indirect immunofluorescence assay after serial		
passage <sup>9</sup>		

<sup>&</sup>lt;sup>1</sup>Cercopithecus aethiops kidney, ATCC<sup>®</sup> CRL-1586™

## /Heather Couch/

**Heather Couch** 29 OCT 2018

Program Manager or designee, ATCC Federal Solutions

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>Source of irradiated antigen: BEI Resources NR-50240 lot 70002764

<sup>&</sup>lt;sup>3</sup>All tests were completed post-inactivation unless specified

<sup>&</sup>lt;sup>4</sup>The Tissue Culture Infectious Dose 50% (TCID<sub>50</sub>) endpoint 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% (LD50) 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>5</sup>7 days at 28°C and 5% CO<sub>2</sub>

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

<sup>&</sup>lt;sup>7</sup>ddPCR data was obtained post-vial from 9 replicates on the BioRad QX200 Droplet Digital PCR (ddPCR™) System

<sup>&</sup>lt;sup>8</sup>Performed at University of Texas Medical Branch, Galveston, Texas, USA

The inactivated virus preparation was plated on Vero E6 cells and incubated for 14 days at 37°C and 5% CO2; cell lysate and supernatant from these cultures was passaged on fresh monolayers of Vero E6 cells and again incubated for 14 days at 37°C and 5% CO<sub>2</sub>.