

## Certificate of Analysis for NR-49850

## Chikungunya Virus, Bianchi

Catalog No. NR-49850

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

**Passage History:** X1V1/V3 (Prior to deposit at BEI Resources/BEI Resources); X# = Number of passages in unknown host; V# = Number of passages in Vero cells<sup>2</sup>

Lot<sup>3</sup>: 64480268 Manufacturing Date: 13OCT2016

TEST	SPECIFICATIONS	RESULTS
Infectivity in Vero E6 Cells	Report results	Cell rounding and detachment
Sequencing of Species-Specific Region (713 nucleotides)	Consistent with CHIKV, Bianchi	100% identity with CHIKV, Bianchi (GenBank: KX262989)
Titer by TCID <sub>50</sub> Assay <sup>4,5</sup> in Vero E6 Cells <sup>1</sup>	Report results	2.8 × 10 <sup>7</sup> TCID <sub>50</sub> per mL
Amplification of CHIKV Sequence by RT-PCR	~ 1070 bp amplicon	~ 1070 bp amplicon
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 extracted 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:** 04 APR 2017

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>The second virus passage at BEI Resources was performed by lipofectamine-mediated transfection of extracted viral nucleic acid in order to remove contaminating mycoplasma.

<sup>&</sup>lt;sup>3</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 and 1.5 g/L of sodium bicarbonate (ATCC® 30-2003) supplemented with 2% fetal bovine serum (ATCC® 30-2020) for 2 days at 37°C with 5% CO<sub>2</sub>

<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% (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>5</sup>4 days at 37°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.