

**Zika Virus, MEX 2-81**

**Catalog No. NR-50280**

**Product Description:** Cell lysate and supernatant from *Cercopithecus aethiops* kidney epithelial cells (Vero 76, clone E6) infected with Zika virus (ZIKV), MEX 2-81

**Passage History:** V4/V3 (Prior to deposit at BEI Resources/BEI Resources); V# = Number of passages in Vero cells<sup>1</sup>

**Lot<sup>2</sup>: 64158308**

**Manufacturing Date: 26APR2016**

TEST	SPECIFICATIONS	RESULTS
<b>Infectivity in Vero E6 Cells</b>	Report results	Refractile cell rounding
<b>Identification by Indirect Fluorescent Antibody (IFA) Assay<sup>3</sup></b>	Fluorescence observed	Fluorescence observed
<b>Sequencing of Species-Specific Region (765 nucleotides)</b>	Consistent with ZIKV	Consistent with ZIKV <sup>4</sup>
<b>Titer by TCID<sub>50</sub> Assay in Vero E6 Cells<sup>5,6</sup> with IFA Readout<sup>3</sup></b>	Report results	8.9 × 10 <sup>6</sup> TCID <sub>50</sub> per mL
<b>Sterility (21-day incubation)</b> 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 No growth No growth No growth No growth No growth	No growth No growth No growth No growth No growth No growth No growth
<b>Mycoplasma Contamination</b> 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>1</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>2</sup>Grown in Minimum Essential Medium containing Earle's Balanced Salt Solution (Gibco® 11095-080), supplemented with 1 mM sodium pyruvate (Gibco® 11360-070) and 2% fetal bovine serum (ATCC® 30-2020) for 7 days at 37°C with 5% CO<sub>2</sub>

<sup>3</sup>Using mouse monoclonal antibody to Zika virus NS1 protein (Aalto Bio Reagents catalog no. AZ 1225, clone 2701106)

<sup>4</sup>Partial nucleotide sequencing of NR-50280 obtained at the time this lot was manufactured is consistent with contemporary ZIKV isolates from the Americas. The complete genomic sequence of NR-50280, Lot No. 64158308 has since been determined and deposited at NCBI (GenBank: KX446950, ZIKV/Aedes.sp/MEX/MEX\_2-81/2016).

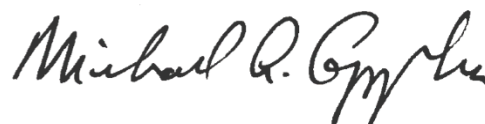
<sup>5</sup>The Tissue Culture Infectious Dose 50% (TCID<sub>50</sub>) is the 50% infectious endpoint in cell culture. The TCID<sub>50</sub> is the dilution of virus that under the conditions of the assay can be expected to infect 50% of the culture vessels inoculated, just as a Lethal Dose 50% (LD<sub>50</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>6</sup>7 days at 37°C with 5% CO<sub>2</sub>

<sup>7</sup>Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

**Date:** 15 MAR 2017

**Signature:**



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