SUPPORTING INFECTIOUS DISEASE RESEARCH

Zika Virus, MR 766

Catalog No. NR-50065

Product Description: Cell lysate and supernatant from *Cercopithecus aethiops* kidney epithelial cells (Vero)¹ infected with Zika virus (ZIKV), MR 766

Passage History: SM150/V8 (Prior to deposit at BEI/BEI); SM# = Number of passages in suckling mice; V# = Number of passages in Vero cells

Lot²: 64254618

Manufacturing Date: 19FEB2016

TEST	SPECIFICATIONS	RESULTS
Infectivity in Vero Cells ¹	Report results	Cell rounding and detachment
Sequencing of Species-Specific Region (840 nucleotides)	Consistent with ZIKV, MR 766	99% identity with ZIKV, MR 766 (GenBank: AY632535) ³
Titer by TCID ₅₀ Assay in Vero Cells by CPE ^{1,4,5}	Report results	2.8 × 10 ⁸ TCID ₅₀ per mL
Sterility (21-day incubation) Harpo's HTYE broth ⁶ , 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 ₂	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
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

¹Vero cells: ATCC[®] CCL-81[™]

²Grown in Dulbecco's Modified Eagle's Medium containing 4 mM L-glutamine, 4500 mg per L glucose, 1 mM sodium pyruvate, and 1500 mg per L sodium bicarbonate (ATCC[®] 30-2002), supplemented with 2% fetal bovine serum (ATCC[®] 30-2020) for 3 days at 37°C with 5% CO₂
³The complete coding sequence of NR-50065, Lot No. 63856750 has also been determined (GenBank: KU963573).

⁴The Tissue Culture Infectious Dose 50% (TCID₅₀) is the 50% infectious endpoint in cell culture. The TCID₅₀ 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 TCID₅₀ provides a measure of the titer (or infectivity) of a virus preparation. ⁵6 days at 37°C with 5% CO₂

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

Date: 11 JUL 2016

Signature:

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