

**Heartland Virus, MO-4**

**Catalog No. NR-49771**

**Product Description:** Cell lysate and supernatant from *Cercopithecus aethiops* kidney epithelial cells (Vero)<sup>1</sup> infected with Heartland virus (HRTV)

**Passage History:** D1V2/V2 (Prior to deposit at BEI/BEI); D# = Number of passages in DH82 cells; V# = Number of passages in Vero cells

**Lot<sup>2</sup>: 63721378**

**Manufacturing Date: 16SEP2015**

TEST	SPECIFICATIONS	RESULTS
<b>Infectivity in Vero Cells<sup>1</sup></b>	Report results	Cell rounding and detachment
<b>Sequencing of Species-Specific Region (387 nucleotides)</b>	Consistent with HRTV	99% identity with HRTV (GenBank: JX005847)
<b>Titer by TCID<sub>50</sub> Assay in Vero Cells With CPE Readout<sup>1,3,4</sup></b>	Report results	1.6 × 10 <sup>7</sup> TCID <sub>50</sub> per mL
<b>Sterility (21-day incubation)</b> Harpo's HTYE broth <sup>5</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>Vero cells: ATCC® CCL-81™

<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® 30-2003) and 2% fetal bovine serum (ATCC® 30-2020) for 6 days at 37°C with 5% CO<sub>2</sub>

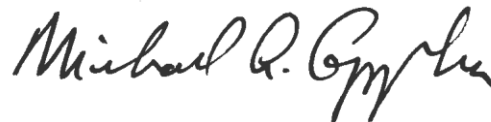
<sup>3</sup>The Tissue Culture Infectious Dose 50% (TCID<sub>50</sub>) is the 50% infectious endpoint in cell culture. The TCID<sub>50</sub> is the dilution of virus that under the conditions of the assay can be expected to infect 50% of the culture vessels inoculated, just as a Lethal Dose 50% (LD<sub>50</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>4</sup>8 days at 37°C with 5% CO<sub>2</sub>

<sup>5</sup>Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

**Date:** 19 NOV 2015

**Signature:**



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