

## **Certificate of Analysis for NR-9382**

## Seoul Hantavirus, Baltimore

## Catalog No. NR-9382

This reagent is the tangible property of the U.S. Government.

**Product Description:** Cell lysate and supernatant from *Cercopithecus aethiops* kidney epithelial cells (Vero E6)<sup>1</sup> infected with Seoul hantavirus, Baltimore

Lot<sup>2</sup>: 63527972 Manufacturing Date: 08DEC2015

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero E6 Cells <sup>1</sup> Using RT-PCR <sup>3</sup>	Report results	Infectious, no cytopathic effect
Sequencing of Species-Specific Region (323 nucleotides)	Consistent with Seoul hantavirus	Consistent with Seoul hantavirus <sup>4</sup>
Titer by TCID <sub>50</sub> Assay <sup>5</sup> in Vero E6 Cells <sup>1</sup> Using RT-PCR <sup>3</sup>	Report results	2.8 x 10 <sup>3</sup> TCID <sub>50</sub> per mL
Amplification of Hantavirus Sequence by RT-PCR	~ 370 bp amplicon	~ 370 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 Test Article nucleic acid	None detected None detected	None detected None detected

<sup>&</sup>lt;sup>1</sup>Vero E6 cells; ATCC® CRL-1586™

<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.

**Date:** 20 DEC 2016

Signature:

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<sup>&</sup>lt;sup>2</sup>Grown in Eagle's Minimum Essential Medium (ATCC® 30-2003™) supplemented with 2% fetal bovine serum (ATCC® 30-2020™) for 14 days at 37°C and 5% CO₂

<sup>&</sup>lt;sup>3</sup>RNA used for RT-PCR was extracted from infected cell lysate and supernatant after 14 days incubation at 37°C and 5% CO<sub>2</sub>.

<sup>&</sup>lt;sup>4</sup>Sequence information for the S segment of Seoul hantavirus, Baltimore is not available in the NCBI database; nucleotide sequence obtained for NR-9382, Lot No. 63527972 is highly similar to several Seoul hantavirus strains.

<sup>&</sup>lt;sup>5</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.