

***Rickettsia rickettsii*, Strain Lost Horse Canyon**

**Catalog No. NR-327**

**Product Description:** Cell lysate and supernatant from African green monkey kidney (Vero) cells<sup>1</sup> infected with *Rickettsia rickettsii*, strain Lost Horse Canyon.

**Lot<sup>2</sup>: 58362750**

**Manufacturing Date: 08DEC2008**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero Cells <sup>1</sup>	Report results	Cell rounding and sloughing
Identification by Sequencing of Citrate Synthase Gene (770 bp)	<i>Rickettsia rickettsii</i>	<i>Rickettsia rickettsii</i>
Titer by TCID <sub>50</sub> Assay <sup>3,4</sup> in Vero Cells <sup>1</sup>	Report results	1.6 X 10 <sup>3</sup> TCID <sub>50</sub> /mL
PCR Amplification of Extracted DNA	~ 1154 bp amplicon	~ 1154 bp amplicon
<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 Brucella 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 No growth No growth
<b>Mycoplasma Contamination</b> Agar and broth culture (30-day incubation at 37°C) DNA detection by PCR of extracted Test Article nucleic acid	None detected None detected	None detected None detected

<sup>1</sup>Vero cells: ATCC<sup>®</sup> CCL-81™

<sup>2</sup>The material deposited by Dr. F. Marilyn Bozeman was grown in Minimum Essential Medium with Earle's salts (Invitrogen™ 10370-021) supplemented with 10% irradiated fetal bovine serum (Lonza 14-471), 2 mM L-glutamine (Invitrogen™ 25030) and 1 mM sodium pyruvate (Invitrogen™ 11360-070) for 18 days at 35°C and 5% CO<sub>2</sub>.

<sup>3</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>4</sup>6 days at 35°C and 5% CO<sub>2</sub> with media overlay

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

**Date:** 14 MAY 2009

**Signature:** Signature on file

**Title:** Technical Manager, BEI Authentication or designee

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