

## Certificate of Analysis for NR-10402

## Rickettsia parkeri, Strain Maculatum C

Catalog No. NR-10402

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

Lot<sup>2</sup>: 58365923 Manufacturing Date: 03DEC2008

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero Cells <sup>1</sup>	Report results	Refractile and degenerate cell rounding
Identification by Sequencing of Citrate Synthase Gene ( ~ 1030 bp)	Rickettsia parkeri	Rickettsia parkeri <sup>3</sup>
Titer by TCID₅₀ Assay⁴₅⁵ in Vero Cells¹	Report results	1.6 X 10 <sup>6</sup> TCID₅₀/mL
PCR Amplification of Extracted DNA	~ 1154 bp amplicon	~ 1154 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 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
Mycoplasma Contamination  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>&</sup>lt;sup>1</sup>Vero cells: ATCC<sup>®</sup> CCL-81™

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**Date:** 11 MAY 2009 **Signature:** Signature on File

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

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<sup>&</sup>lt;sup>2</sup>The material deposited by Dr. Gregory A. Dasch was grown in Minimum Essential Medium with Earle's salts (Invitrogen™ 10370) supplemented with 10% irradiated fetal bovine serum (Lonza 14-471F), 2 mM L-glutamine (Invitrogen™ 25030-081) and 1 mM sodium pyruvate (Invitrogen™ 11360-070) for 14 days at 35°C and 5% CO₂.

<sup>&</sup>lt;sup>3</sup>Also consistent with other *Rickettsia* species

<sup>&</sup>lt;sup>4</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>&</sup>lt;sup>5</sup>8 days at 35°C and 5% CO<sub>2</sub> with media overlay

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