

## **Certificate of Analysis for NR-46447**

## Ehrlichia chaffeensis, Strain Osceola

## Catalog No. NR-46447

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

Product Description: Cell lysate and supernatant from Canis familiaris macrophage-monocyte cells<sup>1</sup> infected with Ehrlichia chaffeensis (E. chaffeensis), strain Osceola, containing 45% fetal bovine serum and 5% DMSO (final concentrations).

Lot<sup>2</sup>: 62795200 Manufacturing Date: 10FEB2016

TEST	SPECIFICATIONS	RESULTS
Identification by Indirect Fluorescent Antibody (IFA) Assay³	Fluorescence observed	Fluorescence observed
Identification by Sequencing of Species-Specific Region (899 nucleotides)	Consistent with <i>E. chaffeensis</i> , strain Osceola	100% identity with <i>E.</i> chaffeensis, strain Osceola (GenBank: CP007477)
Titer by TCID₅ Assay⁴,⁵ in DH82 Cells¹ by IFA³	Report results	8.9 × 10 <sup>6</sup> TCID <sub>50</sub> per mL
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 (14-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>DH82 cells: ATCC® CRL-10389™

Date: 03 JAN 2017

**BEI Resources Authentication** 

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<sup>&</sup>lt;sup>2</sup>Grown in Eagle's Minimum Essential Medium with Earle's salts, non-essential amino acids, L-glutamine and sodium pyruvate (ATCC® 30-2003) supplemented with 5% fetal bovine serum (ATCC® 30-2020, heat inactivated at 56°C for 30min) for 7 days at 37°C and 5% CO<sub>2</sub>. <sup>3</sup>Using *Ehrlichia chaffeensis* IFA IgG reagent kit (Fuller Laboratories ECHG-120)

<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>513</sup> days at 37°C and 5% CO<sub>2</sub>

<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.