

# **Certificate of Analysis for NR-46446**

## Ehrlichia chaffeensis, Strain Liberty

### Catalog No. NR-46446

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

### **Product Description:**

Ehrlichia chaffeensis (E. chaffeensis), strain Liberty was isolated in 1998 from the blood of a patient in Florida, USA, with a suspected diagnosis of human monocytic ehrlichiosis (HME). NR-46446 lot 70028935 was produced by infecting DH82 cells (ATCC® CRL-10389™) and incubating in Dulbecco's Modified Eagle's Medium (DMEM) containing 4 mM L-glutamine, 4500 mg per L glucose, 1 mM sodium pyruvate and 1500 mg per L sodium bicarbonate (ATCC® 30-2002™), supplemented with 7% fetal bovine serum (ATCC® 30-2020™) that was heat-inactivated and an additional 2 mM L-glutamine (ATCC® 30-2214™) for 3 days at 37°C with 5% CO₂. NR-46446 is provided in cell lysate and supernatant supplemented with 45% heat-inactivated fetal bovine serum and 5% DMSO.

Lot: 70028935 Manufacturing Date: 13SEP2019

TEST	SPECIFICATIONS	RESULTS
Identification of Infectivity in DH82 Cells by Indirect Fluorescent Antibody (IFA) Assay <sup>1</sup>	Fluorescence observed	Fluorescence observed
Genotypic Analysis	> 000/ id-artity with	4000/ :
Sequencing of <i>ECH_0849</i> gene (~ 920 base pairs)	≥ 98% identity with  E. chaffeensis, strain Liberty (GenBank: CP007476.1)	100% identity with  E. chaffeensis, strain Liberty (GenBank: CP007476.1)
Titer by TCID₅ Assay in DH82 Cells by IFA¹,²	Report results	$8.9 \times 10^5$ TCID <sub>50</sub> per mL in 7 days at 37°C with 5% CO <sub>2</sub>
Sterility (21-day incubation)		
Harpo's HTYE broth, 37°C and 26°C, aerobic <sup>3</sup>	No growth	No growth
Trypticase soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud broth, 37°C and 26°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, anaerobic	No growth	No growth
Thioglycollate broth, 37°C, anaerobic	No growth	No growth
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)	None detected	None detected
DNA detection by PCR of extracted Test Article nucleic acid	None detected	None detected

<sup>&</sup>lt;sup>1</sup>Ehrlichia chaffeensis IFA IgG reagent kit (Fuller Laboratories ECHG-120)

#### /Heather Couch/

Heather Couch 25 MAR 2020

Program Manager or designee, ATCC Federal Solutions

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<sup>&</sup>lt;sup>2</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 organism 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 the organism preparation.

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