

# **Certificate of Analysis for NR-48593**

### Anaplasma phagocytophilum, Strain HGE2

#### Catalog No. NR-48593

#### **Product Description:**

Anaplasma phagocytophilum (A. phagocytophilum), strain HGE2 was isolated from a human in Minnesota, USA. NR-48593 lot 70026735 was produced by infecting HL-60 cells (ATCC<sup>®</sup> CCL-240<sup>™</sup>) and incubating in RPMI-1640 medium containing 10% fetal bovine serum (ATCC<sup>®</sup> 30-2020) for 5 days at 37°C with 5% CO<sub>2</sub>.

Lot: 70026735 Manufacturing Date: 22JUL2019

TEST	SPECIFICATIONS	RESULTS
Identification by Indirect Fluorescent Antibody (IFA) Assay <sup>1</sup>	Fluorescence observed	Fluorescence observed
Genotypic Analysis Sequencing of 16S ribosomal RNA gene (~ 1300 base pairs)	≥ 99% identity with  A. phagocytophilum, strain HGE2 (GenBank: LAOE01000001.1)	100% identity with  A. phagocytophilum, strain HGE2 (GenBank: LAOE01000001.1)²
Titer by TCID₅₀ Assay in HL-60 Cells by IFA¹,³	Report results	8.9 × 10 <sup>3</sup> TCID <sub>50</sub> per mL in 9 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>4</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>A. phagocytophilum IFA IgG reagent kit (Fuller Laboratories EEG-120)

## /Heather Couch/

Heather Couch 27 MAR 2020

Program Manager or designee, ATCC Federal Solutions

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<sup>&</sup>lt;sup>2</sup>Also consistent with Ehrlichia equi and "HGE agent," however, these species were recently recognized as A. phagocytophilum. For more information, please see Dumler, J. S., et al. "Reorganization of Genera in the Families Rickettsiaceae and Anaplasmataceae in the Order Rickettsiales: Unification of Some Species of Ehrlichia with Anaplasma, Cowdria with Ehrlichia and Ehrlichia with Neorickettsia, Descriptions of Six New Species Combinations and Designation of Ehrlichia equi and 'HGE agent' as Subjective Synonyms of Ehrlichia phagocytophila." Int. J. Syst. Evol. Microbiol. 51 (2001): 2145-2165. PubMed: 11760958.

<sup>&</sup>lt;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 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>4</sup>Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798