

Certificate of Analysis for NR-48807

Anaplasma phagocytophilum, Strain NCH-1

Catalog No. NR-48807

Product Description:

Anaplasma phagocytophilum (A. phagocytophilum), strain NCH-1 was isolated in 1997 from the blood of a patient suffering from human granulocytic ehrlichiosis in Massachussetts, USA. NR-48807 was produced by infecting HL-60 cells with the seed lot and incubating in RPMI-1640 medium containing 2 mM L-glutamine, 10 mM HEPES, 4500 mg/L glucose, 1 mM sodium pyruvate and 1500 mg/L sodium bicarbonate (ATCC® 30-2001) supplemented with 10% fetal bovine serum (ATCC® 30-2020) for 3 days at 37°C with 5% CO₂. NR-48807 was vialed in RPMI-1640 medium supplemented with 10% fetal bovine serum and 12% DMSO (final concentrations).

Lot: 70036996 Manufacturing Date: 20JUL2020

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity of HL-60 Cells¹ by Indirect Fluorescent Antibody (IFA) Assay²	Fluorescence observed	Fluorescence observed
Genotypic Analysis		
Sequencing of 16S ribosomal RNA gene (~ 1410 base pairs)	≥ 99.9% identity with A. phagocytophilum, strain NCH-1 (GenBank: LANT01000009.1)	99.9% identity with A. phagocytophilum, strain NCH-1 (GenBank: LANT01000009.1) ³
Titer by TCID ₅₀ Assay ^{4,5} in HL-60 Cells¹ by IFA² 8 days at 37°C with 5% CO2	Report results	2.8 × 10 ⁵ TCID ₅₀ per mL
Sterility (21-day incubation)		
Harpo's HTYE broth, 37°C and 26°C, aerobic ⁶	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 ₂	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

¹Human promyelocytic leukemia cells (HL-60; ATCC[®] CCL-240™)

/Heather Couch/

Heather Couch 18 NOV 2020

Program Manager or designee, ATCC Federal Solutions

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²Using A. phagocytophilum IFA IgG reagent kit (Fuller Laboratories EEG-120)

³Also 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.

⁴The Tissue Culture Infectious Dose 50% (TCID₅₀) endpoint is the 50% infectious endpoint in cell culture. The TCID₅₀ 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% (LD₅₀) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the TCID₅₀ provides a measure of the titer (or infectivity) of a virus preparation. ⁵8 days at 37°C and 5% CO₂

⁶Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.