

Certificate of Analysis for NR-46500

Naegleria fowleri, Strain CDC:V597

Catalog No. NR-46500

Product Description: *Naegleria fowleri (N. fowleri)*, strain CDC:V597 is a clinical isolate isolated in 2007 from the cerebral spinal fluid of a 10-year-old male patient in the United States.

Lot¹: 63445971 Manufacturing Date: 30APR2015

TEST	SPECIFICATIONS	RESULTS
Genotyping Sequencing of Internal Transcribed Spacer 1 (ITS 1) and 5.8S ribosomal RNA gene (~ 530 base pairs)	Consistent with N. fowleri	Consistent with <i>N. fowleri</i> , genotype I ²
Functional Activity by PCR Amplification ³ ITS 1, 5.8S ribosomal RNA gene	~ 600 base pair amplicon	~ 600 base pair amplicon
Viable Cell Count by Hemacytometry (pre-freeze)	> 10 ⁶ cells/mL	~ 7 x 10 ⁶ cells/mL
Viability (post-freeze) ⁴	Growth	Growth
Sterility (21-day incubation) Harpo's HTYE broth ⁵ , 37°C and 26°C, aerobic Trypticase soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic Brain heart infusion, 37°C and 26°C, aerobic Sheep blood agar, 37°C, aerobic Sheep blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic	No growth	No growth

NR-46500 was produced by cultivation of the deposited material in modified PYNFH medium (ATCC® medium 1034) supplemented with 10% heat-inactivated fetal bovine serum for 3 days at 35°C in an aerobic atmosphere until peak density was reached.

Date: 28 JUL 2015 Signature:

BEI Resources Authentication

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

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²For genotyping details refer to Zhou, L., et al. "Genetic Variations in the Internal Transcribed Spacer and Mitochondrial Small Subunit rRNA Gene of *Naegleria* Spp." <u>J. Eukaryot. Microbiol.</u> 50 (2003): 522-526. PubMed: 14736150.

³PCR amplification was performed using the NF-ITŚ-F1 and NT-ITS-F2 primer set as described in Zhou, L., et al. "Genetic Variations in the Internal Transcribed Spacer and Mitochondrial Small Subunit rRNA Gene of *Naegleria* Spp." <u>J. Eukaryot. Microbiol.</u> 50 (2003): 522-526. PubMed: 14736150.

⁴Viable cells were observed after 2 days under cultivation conditions.

⁵Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.