

Certificate of Analysis for NR-46499

Naegleria fowleri, Strain CDC:V596

Catalog No. NR-46499

Product Description: Naegleria fowleri (N. fowleri), strain CDC:V596 is a clinical isolate collected in 2007 from the cerebral spinal fluid of a male patient.

Lot¹: 62892081 Manufacturing Date: 22AUG2014

TEST	SPECIFICATIONS	RESULTS
Genotyping Sequencing of Internal Transcribed Spacer 1 (ITS 1) and 5.8S ribosomal RNA gene (~ 580 base pairs)	Consistent with N. fowleri	Consistent with <i>N. fowleri</i> , genotype III 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	5.5 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-46499 was produced by cultivation of the deposited material in modified PYNFH medium (ATCC[®] medium 1034) supplemented with 10% heat-inactivated fetal bovine serum for 4 days at 35°C in an aerobic atmosphere until peak density was reached.

Date: 11 NOV 2014

Signature:

Title: Technical Manager, BEI Authentication or designee

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BEI Resources www.beiresources.org E-mail: contact@beiresources.org
Tel: 800-359-7370

Tel: 800-359-7370 Fax: 703-365-2898

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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." J. Eukaryot. Microbiol. 50 (2003): 522-526. PubMed: 14736150.

³PCR amplification was performed using the NF-ITS-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." J. Eukaryot. Microbiol. 50 (2003): 522-526. PubMed: 14736150.

⁴Viable cells were observed after 1 day 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.