

Certificate of Analysis for NR-46498

Naegleria fowleri, Strain CDC:V566

Catalog No. NR-46498

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

Product Description: *Naegleria fowleri (N. fowleri)*, strain CDC:V566 is a clinical isolate collected in 2006 from the brain of a 7-year-old male in Arizona, USA.

Lot¹: 70004513 Manufacturing Date: 17APR2017

TEST	SPECIFICATIONS	RESULTS
Cellular Morphology ²	Report results	Polymorphic and refractile
Genotyping³ Sequencing of Internal Transcribed Spacer 1 (ITS), 5.8S ribosomal RNA (rRNA) gene, ITS 2 (~ 530 base pairs)	Consistent with N. fowleri	Consistent with <i>N. fowleri</i> (genotype I) ^{4,5}
Functional Activity by PCR Amplification ^{3,6} ITS 1, 5.8S ribosomal RNA gene, ITS2	~ 600 base pair amplicon	~ 600 base pair amplicon
Viable Cell Count by Hemacytometry ³	> 10 ⁶ cells/mL	2.0 × 10 ⁷ cells/mL
Viability ^{2,7}	Growth	Growth
Sterility (21-day incubation) ² Harpo's HTYE broth ⁸ , 37°C and 26°C, aerobic Trypticase soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic DMEM with 10% FBS, 37°C, aerobic Sheep blood agar, 37°C, aerobic Sheep blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic	No growth	No growth

¹NR-46498 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: 11 AUG 2017

www.beiresources.org

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.

ATCC® is a trademark of the American Type Culture Collection.

You are authorized to use this product for research use only. It is not intended for human use.

BEI Resources E-mail: contact@beiresources.org

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

²Testing completed on vialed, post-freeze material.

³Testing completed on bulk material prior to vialing and freezing.

⁴For 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.

⁵Also consistent with *Naegleria Iovaniensis*

⁶PCR 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." <u>J. Eukaryot. Microbiol.</u> 50 (2003): 522-526. PubMed: 14736150.

⁷Viable cells were observed after 1 day at 35°C in an aerobic atmosphere.

⁸Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.