

Certificate of Analysis for NR-46505

Naegleria fowleri, Strain CDC:V626

Catalog No. NR-46505

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

Product Description: *Naegleria fowleri (N. fowleri)*, strain CDC:V626 is a clinical isolate collected in 2010 from the cerebral spinal fluid of a 7-year-old female.

Lot^{1,2}: 2170 Manufacturing Date: 01NOV2016

TEST	SPECIFICATIONS	RESULTS
Cellular Morphology	Report results	Refractile
Genotyping Sequencing of Internal Transcribed Spacer 1 (ITS 1) and 5.8S ribosomal RNA gene (~ 590 base pairs)	Consistent with N. fowleri	Consistent with <i>N. fowleri</i> , genotype III ³
Functional Activity by PCR Amplification ⁴ 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	3 x 10 ⁶ cells/mL
Viability⁵	Growth	Growth
Sterility (21-day incubation) Harpo's HTYE broth ⁶ , 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

NR-46505 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: 29 MAR 2017 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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BEI Resources

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²Quality control testing completed on post-freeze material unless specified as pre-freeze.

³For 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.

⁴PCR 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.

⁵Viable cells were observed after 1 day in modified PYNFH medium supplemented with 10% heat-inactivated fetal bovine serum at 35°C in an aerobic atmosphere.

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