

Certificate of Analysis for NR-46507

Naegleria fowleri, Strain CDC:V631

Catalog No. NR-46507

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

Product Description: *Naegleria fowleri (N. fowleri)*, strain CDC:V631 is a clinical isolate collected in 2011 from the cerebral spinal fluid of a 28-year-old male in Louisiana, USA.

Lot^{1,2}: 2238 Manufacturing Date: 28NOV2016

TEST	SPECIFICATIONS	RESULTS
Cellular Morphology ²	Report results	Adherent and refractile
Genotyping ³ Sequencing of Internal Transcribed Spacer 1 (ITS 1) and 5.8S ribosomal RNA gene (~ 540 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	~ 600 base pair amplicon	~ 600 base pair amplicon
Viable Cell Count by Hemacytometry ³	> 10 ⁶ cells/mL	1.34 x 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-46507 was produced by cultivation of the deposited material in modified PYNFH medium (ATCC® medium 1034) supplemented with 10% heat-inactivated fetal bovine serum for 5 days at 35°C in an aerobic atmosphere until peak density was reached.

Date: 16 MAY 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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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." J. Eukaryot. Microbiol. 50 (2003): 522-526. PubMed: 14736150.

⁷Viable cells were observed after 1 day under cultivation conditions.

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