

Certificate of Analysis for NR-46502

Naegleria fowleri, Strain CDC:V616

Catalog No. NR-46502

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

Product Description: *Naegleria fowleri (N. fowleri)*, strain CDC:V616 is a clinical isolate collected in 2009 from the cerebral spinal fluid of a 22-year-old male in the United States.

Lot^{1,2}: 64463930 Manufacturing Date: 02AUG2016

| TEST | SPECIFICATIONS | RESULTS |
|--|---|---|
| Genotyping Sequencing of Internal Transcribed Spacer 1 (ITS 1) and 5.8S ribosomal RNA gene (~ 550 base pairs) | Consistent with N. fowleri | Consistent with <i>N. fowleri</i> , genotype I 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 ⁷ cells/mL |
| Viability (post-freeze) ⁵ | 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 |

¹NR-46502 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: 03 FEB 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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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." J. Eukaryot. Microbiol. 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 under cultivation conditions.

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