

## **Certificate of Analysis for NR-46489**

## Naegleria fowleri, Strain CDC:V020

## Catalog No. NR-46489

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

**Product Description:** *Naegleria fowleri (N. fowleri)*, strain CDC:V020 is a clinical isolate collected in 1984 from the cerebral spinal fluid of a 12-year-old male in Texas, USA.

Lot<sup>1,2</sup>: 70002373 Manufacturing Date: 23MAR2017

TEST	SPECIFICATIONS	RESULTS
Cellular Morphology <sup>2</sup>	Report results	Adherent and refractile
Genotyping³ Sequencing of Internal Transcribed Spacer 1 (ITS), 5.8S ribosomal RNA (rRNA) gene, ITS 2 (~ 540 base pairs)	Consistent with N. fowleri	Consistent with <i>N. fowleri</i> (genotype I) <sup>4,5</sup>
Functional Activity by PCR Amplification <sup>3,6</sup> ITS 1, 5.8S ribosomal RNA gene, ITS2	~ 600 base pair amplicon	~ 600 base pair amplicon
Viable Cell Count by Hemacytometry <sup>3</sup>	> 10 <sup>6</sup> cells/mL	5.55 × 10 <sup>6</sup> cells/mL
Viability <sup>2,7</sup>	Growth	Growth
Sterility (21-day incubation) <sup>2</sup> Harpo's HTYE broth <sup>8</sup> , 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

<sup>&</sup>lt;sup>1</sup>NR-46489 was produced by cultivation of the deposited material in modified PYNFH medium (ATCC® medium 1034) supplemented with 10% heat-inactivated fetal bovine serum for 13 days at 35°C in an aerobic atmosphere until peak density was reached.

Date: 16 AUG 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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<sup>&</sup>lt;sup>2</sup>Testing completed on vialed, post-freeze material.

<sup>&</sup>lt;sup>3</sup>Testing completed on bulk material prior to vialing and freezing.

<sup>&</sup>lt;sup>4</sup>For 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.

<sup>&</sup>lt;sup>5</sup>Also consistent with *Naegleria Iovaniensis* 

<sup>&</sup>lt;sup>6</sup>PCR 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.

<sup>&</sup>lt;sup>7</sup>Viable cells were observed after 1 day at 35°C in an aerobic atmosphere.

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