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SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-46491

Naegleria fowleri, Strain CDC:V204

Catalog No. NR-46491

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

Product Description: *Naegleria fowleri (N. fowleri)*, strain CDC:V204 is a clinical isolate collected in 1990 from the cerebrospinal fluid of a male in Mexico.

Lot[1]: 70006374 **Manufacturing Date: 09JUN2017**

TEST	SPECIFICATIONS	RESULTS
Cellular Morphology[2]	Report results	Polymorphic and refractile
Genotyping[3] Sequencing of Internal Transcribed Spacer 1 (ITS), 5.8S ribosomal RNA (rRNA) gene, ITS 2 (~ 530 base pairs)	Consistent with *N. fowleri*	Consistent with *N. fowleri* (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	1.02 × 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 No growth No growth No growth No growth No growth	 No growth No growth No growth No growth No growth No growth No growth

[1]NR-46491 was produced by cultivation of the deposited material in modified PYNFH medium (ATCC® medium 1034) supplemented with 10% heat-inactivated fetal bovine serum for 2 days at 35°C in an aerobic atmosphere until peak density was reached.
[2]Testing completed on vialed, post-freeze material.
[3]Testing completed on bulk material prior to vialing and freezing.
[4]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." J. Eukaryot. Microbiol. 50 (2003): 522-526. PubMed: 14736150.
[5]Also consistent with *Naegleria lovaniensis*
[6]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." J. Eukaryot. Microbiol. 50 (2003): 522-526. PubMed: 14736150.
[7]Viable cells were observed after 1 day under cultivation conditions.
[8]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Date: 22 JAN 2018 **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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