

## **Certificate of Analysis for NR-46468**

## Acanthamoeba sp., Strain CDC:V333

## Catalog No. NR-46468

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

**Product Description:** Acanthamoeba sp., strain CDC:V333 is a clinical isolate collected in 1995 from the brain tissue of a male patient in Georgia, USA. Strain CDC:V333 was deposited to BEI Resources as genotype T1 based on 18S ribosomal RNA gene sequence analysis.

Lot<sup>1,2</sup>: 64357370 Manufacturing Date: 22JUN2016

TEST	SPECIFICATIONS	RESULTS
Cellular Morphology	Report results	Adherent and non-adherent
Genotyping Sequencing of 18S ribosomal RNA gene (250 base pairs)	≥ 99% sequence identity to  Acanthamoeba sp.,  strain CDC:V333  (GenBank: FJ196644.2)	100% sequence identity to Acanthamoeba sp., strain CDC:V333 (GenBank: FJ196644.2)
Functional Activity by PCR Amplification <sup>3</sup> 18S ribosomal RNA gene (amplicon ASA.S1)	423 to 551 base pair amplicon	~ 450 base pair amplicon
Viable Cell Count by Hemocytometry (pre-freeze)	> 10 <sup>6</sup> cells/mL	5.4 x 10 <sup>6</sup> cells/mL
Viability <sup>4</sup>	Growth	Growth
Sterility (21-day incubation) Harpo's HTYE broth <sup>5</sup> , 37°C and 26°C, aerobic Tryptic Soy broth, 37°C and 26°C, aerobic Sabouraud Dextrose 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-46468 was produced by cultivation of the deposited material in Peptone Yeast Glucose medium (PYG; ATCC® medium 712) for 5 days at 30°C in an aerobic atmosphere to produce this lot.

**Date:** 06 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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<sup>&</sup>lt;sup>2</sup>Quality control testing completed on post-freeze material unless specified as pre-freeze.

<sup>&</sup>lt;sup>3</sup>PCR amplification was performed using the JDP1 and JDP2 primer set (JDP1: 5'-GGCCCAGATCGTTTACCGTGAA-3' and JDP2: 5'-TCTCACAAGCTGCTAGGGAGTCA-3') as described in Schroeder, J. M., et al. "Use of Subgenic 18S Ribosomal DNA PCR and Sequencing for Genus and Genotype Identification of Acanthamoebae from Humans with Keratitis and from Sewage Sludge." J. Clin. Microbiol. 39 (2001): 1903-1911. PubMed: 11326011.

<sup>&</sup>lt;sup>4</sup>Viable cells were observed after 1 day at 30°C in an aerobic atmosphere in PYG medium.

<sup>&</sup>lt;sup>5</sup>Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.