

Certificate of Analysis for NR-46473

Acanthamoeba sp., Strain CDC:V522

Catalog No. NR-46473

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

Product Description: Acanthamoeba sp., strain CDC:V522 (also referred to as OSU 03-035) is a clinical isolate collected in 2003 from the skin tissue of a male patient in Massachusetts, USA.

Lot¹: 64508702 Manufacturing Date: 02SEP2016

TEST	SPECIFICATIONS	RESULTS
Cellular Morphology ²	Report results	Adherent and non-adherent
Genotyping ³ Sequencing of 18S ribosomal RNA gene (~ 290 base pairs)	≥ 99% sequence identity to Acanthamoeba sp., strain CDC:V522 (GenBank: AY703016.1)	100% sequence identity to Acanthamoeba sp., strain CDC:V522 (GenBank: AY703016.1)
Functional Activity by PCR Amplification ^{3,4} 18S ribosomal RNA gene (amplicon ASA.S1)	423 to 551 base pair amplicon	~ 450 base pair amplicon
Viable Cell Count by Hemocytometry ³	> 10 ⁶ cells/mL	$7.0 \times 10^6 \text{ cells/mL}$
Viability ^{2.5}	Growth	Growth
Sterility (21-day incubation) ² Harpo's HTYE broth ⁶ , 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

¹NR-46473 was produced by cultivation of the deposited material in Peptone Yeast Glucose (PYG) medium (ATCC® medium 712) for 4 days at 30°C in an aerobic atmosphere to produce this lot.

Date: 03 APR 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.

⁴PCR 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." <u>J. Clin. Microbiol.</u> 39 (2001): 1903-1911. PubMed: 11326011.

⁵Viable cells were observed after 2 days at 30°C in an aerobic atmosphere in PYG medium.

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