

Certificate of Analysis for NR-42489

Leishmania major, Strain NIH SD (MHOM/SN/74/SD), ∆gp63 1-7

Catalog No. NR-42489

Product Description: Leishmania major (L. major), strain NIH SD (MHOM/SN/74/SD) $\Delta gp63$ 1-7 is a deletion mutant of the wild-type strain NIH SD (MHOM/SN/74/SD) created by the targeted deletion of the seven tandemly-linked genes comprising the leishmanolysin (gp63) gene complex.

Lot¹: 61775804 Manufacturing Date: 23MAY2013

TEST	SPECIFICATIONS	RESULTS
	SPECIFICATIONS	RESULIS
Genotyping Sequencing of 18S ribosomal RNA gene and internal transcribed spacer (ITS) 1 (~ 1540 base pairs) Sequencing of ITS 1, 5.8S ribosomal RNA gene, ITS 2 (~ 670 base pairs)	Consistent with <i>L. major</i> Consistent with <i>L. major</i>	Consistent with <i>L. major</i> ² Consistent with <i>L. major</i>
Functional Activity by PCR Amplification 18S ribosomal RNA gene ITS 1, 5.8S ribosomal RNA gene, ITS 2	~ 2500 base pair amplicon ~ 1200 base pair amplicon	~ 2500 base pair amplicon ~ 1200 base pair amplicon
Presence/Absence of Genes Confirmed by PCR Amplification Leishmanolysin gene 1 (<i>gp63-1</i>) NR-42489 Leishmanolysin gene 1 (<i>gp63-1</i>) positive control	No amplicons ~ 320 base pair amplicon	No amplicons ~ 320 base pair amplicon
Viable Cell Count by Hemacytometry (pre-freeze)	> 10 ⁶ cells/mL	7.8 x 10 ⁷ cells/mL
Viability (post-freeze) ³	Growth	Growth
Sterility (21-day incubation) Harpo's HTYE broth ⁴ , 37°C and 26°C, aerobic Trypticase soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic Brain heart infusion, 37°C and 26°C, aerobic Sheep blood agar, 37°C, aerobic Sheep blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic	No growth	No growth

NR-42489 was produced by cultivation of the deposited. After several low growth passages, the culture was propagated in Modified M199 medium supplemented with hemin for 2 days at 25°C in an aerobic atmosphere to produce this lot.

Date: 16 MAY 2014

Signature: \

Title:

Technical Manager, BEI Authentication or designee

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www.beiresources.org

E-mail: contact@beiresources.org

Tel: 800-359-7370 Fax: 703-365-2898

NR-42489 61775804 16MAY2014

²Also consistent with other *Leishmania* species

³Viable cells were observed after 1 day under cultivation conditions.

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