

Certificate of Analysis for NR-42490

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

Catalog No. NR-42490

Product Description: Leishmania major (L. major), strain NIH SD (MHOM/SN/74/SD) $\Delta gp63$ 1-7 + gp63-1 is a mutant of the wild-type strain NIH SD (MHOM/SN/74/SD) created by the targeted deletion of the leishmanolysin (gp63) gene complex followed by transfection with plasmid pLEXNeo-gp63-1 containing the gp63-1 gene. Complementation mutants were selected from G418-resistant promastigotes and are reported to be resistant to hygromycin B, nourseothricin and neomycin.

Lot¹: 61695012 Manufacturing Date: 10APR2013

TEST	SPECIFICATIONS	RESULTS
Genotyping		
Sequencing of 18S ribosomal RNA gene and internal	Consistent with L. major	Consistent with L. major ²
transcribed spacer (ITS) 1 (~ 1510 base pairs)		
Sequencing of ITS 1, 5.8S ribosomal RNA gene, ITS 2	Consistent with L. major	Consistent with L. major
(~ 660 base pairs)	_	
Functional Activity by PCR Amplification		
18S ribosomal RNA gene	~ 2500 base pair amplicon	~ 2500 base pair amplicon
ITS 1, 5.8S ribosomal RNA gene, ITS 2	~ 1200 base pair amplicon	~ 1200 base pair amplicon
Presence/Absence of Genes Confirmed by PCR Amplification	, ,	
Leishmanolysin gene 1 (<i>gp63-1</i>) NR-42490	~ 320 base pair amplicon	~ 320 base pair amplicon
Leishmanolysin gene 1 (gp63-1) negative control	No amplicons	No amplicons
Viable Cell Count by Hemacytometry	406 11 / 1	7 0 10 ⁷ 11 1
(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	No growth	No growth
Trypticase soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud broth, 37°C and 26°C, aerobic	No growth	No growth
Brain heart infusion, 37°C and 26°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, anaerobic	No growth	No growth
Thioglycollate broth, 37°C, anaerobic	No growth	No growth

¹NR-42490 was produced by cultivation of the deposited. After several low growth passages, the culture was propagated in Modified M199 medium supplemented with hemin for 5 days at 25°C in an aerobic atmosphere to produce this lot.

Date: 19 MAY 2014

Signature:

Title: Technical Manager, BEI Authentication or designee

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²Also consistent with other *Leishmania* species

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

⁴Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.