b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-50182

Leishmania donovani, Strain 1S2D (+*luc*)

Catalog No. NR-50182

Product Description:

Leishmania donovani (*L. donovani*), strain 1S2D (+*luc*) is a transgenic clone derived from strain 1S2D (MHOM/SD/62/1S-CL2D), which was originally isolated in 1962 from a human patient with visceral leishmaniasis in Sudan. NR-50182 lot 64233617 was produced by cultivation of the deposited material in Modified M199 Medium (M199) with Hanks' salts supplemented with 10% heat-inactivated fetal bovine (HIFBS) serum and 10 µg/mL hemin for 4 days at 25°C in an aerobic atmosphere to produce this lot. Note: Culture color changes from bright red to orange during growth of this organism.

Lot: 64233617 **Manufacturing Date: 06MAY2016**

TEST	SPECIFICATIONS	RESULTS
Cell Morphology[1] 1 day at 25°C in an aerobic atmosphere in M199 with Hanks' salts supplemented with 10% HIFBS and 10 µg/mL hemin	Report results	Elongated, refractile, motile
Genotypic Analysis[2] Sequencing of internal transcribed spacer (ITS) 1, 5.8S ribosomal RNA gene, ITS 2 (~ 1040 base pairs)	Consistent with *L. donovani* complex	Consistent with *L. donovani* complex[3]
Functional Activity of Luciferase Gene[2,4]	Positive	Positive
Viable Cell Count by Hemacytometry[2]	> 10^6 cells per mL	3.6 x 10^8 cells/mL
Viability[1] 1 day at 25°C in an aerobic atmosphere in M199 with Hanks' salts supplemented with 10% HIFBS and 10 µg/mL hemin	Growth	Growth
Sterility (21-day incubation)[1]		
Harpo's HTYE broth, 37°C and 26°C, aerobic[5]	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
DMEM with 10% FBS, 37°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

[1]Testing completed on vialed, post-freeze material
[2]Testing completed on bulk material prior to vialing and freezing
[3]*L.* donovani complex consists of three species: *donovani, infantum* and *chagasi* which are not differentiated by this assay. Mauricio, I.L., et al. "Genomic Diversity in the *Leishmania donovani* Complex." Parasitology 119 (1999): 237-246. PubMed: 10503249.
[4]Luciferase activity was determined using the *Renilla* Luciferase Assay System (Promega E2810). Parasites were lysed and incubated with luciferase assay reagent. Luciferase activity was measured using a luminometer with a bioluminescence emission spectra of 480 nm. [Roy, G., et al. "Episomal and Stable Expression of the Luciferase Reporter Gene for Quantifying *Leishmania* spp. Infections in Macrophages and in Animal Models." Mol. Biochem. Parasitol. 110 (2000): 195-206. PubMed: 11071276.].
[5]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

/Heather Couch/
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Program Manager or designee, ATCC Federal Solutions

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