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 was produced by cultivation of BEI Resources seed lot 64233618 in Modified M199 Medium (M199) supplemented with 10% heat-inactivated fetal bovine (HIFBS) serum and 10 µg/mL hemin for 4 days at 25°C in an aerobic atmosphere with the cap screwed on tightly to produce this lot.

Lot: 70048201 **Manufacturing Date: 20SEP2022**

TEST	SPECIFICATIONS	RESULTS
Cell Morphology[1] 2 days at 25°C in an aerobic atmosphere in M199 supplemented with 10% HIFBS and 10 µg/mL hemin	Report results	Elongated, refractile, motile, rosettes visible (Figure 1)
Genotypic Analysis[2] Sequencing of internal transcribed spacer (ITS) 1, 5.8S ribosomal RNA gene, ITS 2 (~ 1010 base pairs) Sequencing of N-acetylglucosamine-1-phosphate transferase gene *(nagt)* (~ 1380 base pairs)	≥ 99% sequence identity to *L. donovani*, Strain Pasteur (GenBank: CP022642.1) Consistent with *L. donovani*	99.9% sequence identity to *L. donovani*, Strain Pasteur (GenBank: CP022642.1)[3] Consistent with *L. donovani*[4]
Functional Activity of Luciferase Gene[2,5]	Positive	Positive
Viable Cell Count by Hemacytometry[2]	> 10^6 cells per mL	3.4 × 10^8 cells/mL
Viability[1] 2 days at 25°C in an aerobic atmosphere in M199 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[6]	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]Also consistent with other members of the *L.* donovani complex, consisting 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]Waki, K., et al. "Transmembrane Molecules for Phylogenetic Analyses of Pathogenic Protists: *Leishmania*-Specific Informative Sites in Hydrophilic Loops of Trans-Endoplasmic Reticulum N-Acetylglucosamine-1-Phosphate Transferase." Eukaryot. Cell. 6 (2007): 198-210. PubMed: 17142569.
[5]Luciferase activity was determined using the Luciferase Assay System (Promega E1500). Parasites were lysed and incubated with luciferase assay reagent. Luciferase activity was measured using a luminometer with a bioluminescence emission spectra of approximately 560 nm.
[6]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

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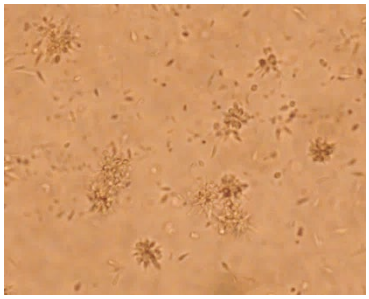
Figure 1: Colony Morphology



/Sonia Bjorum Brower/
Sonia Bjorum Brower 15 NOV 2023
Technical Manager or designee, ATCC Federal Solutions

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