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SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-50608

Leishmania braziliensis, Strain HOM/BR/75/M2903

Catalog No. NR-50608

Product Description:

Leishmania braziliensis (L. braziliensis), strain HOM/BR/75/M2903 was isolated in 1975 from the cutaneous lesion of a male in Para, Brazil. The deposited material was inoculated into Medium 199 (M199) with Hanks' salts supplemented with 10% heat-inactivated fetal bovine serum (HIFBS) and 10 µg/mL hemin and grown for 8 days at 25°C in an aerobic atmosphere, and the resulting subculture was vialed and frozen. NR-50608 was produced by inoculation of the frozen subculture into M199 with Hanks' salts supplemented with 10% HIFBS and 10 µg/mL hemin for 4 days at 25°C in an aerobic atmosphere to produce this lot.

Lot: 70033209 **Manufacturing Date: 25FEB2020**

TEST	SPECIFICATIONS	RESULTS
Cell Morphology[1] 2 days at 25°C in an aerobic atmosphere in M199 with Hanks' salts supplemented with 10% HIFBS and 10 µg/mL hemin	Report results	Ovoid-to-elongated; rosettes visible
Genotypic Analysis[2] Sequencing of internal transcribed spacer (ITS) 1, 5.8S ribosomal RNA gene, ITS 2 (~ 270 base pairs) Sequencing of N-acetylglucosamine-1-phosphate transferase gene *(nagt)* (1300 base pairs)	≥ 99% sequence identity to *L. braziliensis* ≥ 99% sequence identity to *L. braziliensis* var. 4 *nagt* gene (GenBank: DQ836162.1)	100% sequence identity to *L. braziliensis* 99.9% sequence identity to *L. braziliensis* var. 4 *nagt* gene (GenBank: DQ836162.1)[3]
Viable Cell Count by Hemacytometry[1]	> 10^6 cells per mL	3.1 × 10^8 cells per mL
Viability[1] 2 days 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[4] Trypticase soy broth, 37°C and 26°C, aerobic Sabouraud 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 No growth No growth No growth No growth No growth	 No growth No growth No growth No growth No growth 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. braziliensis* var. 4 is differentiated from *L. braziliensis* based on a 4-nucleotide difference in the *nagt* gene (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.).
[4]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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