

Certificate of Analysis for NR-50602

Leishmania donovani, Strain HOM/IN/83/AG83

Catalog No. NR-50602

Product Description:

Leishmania donovani (L. donovani), strain HOM/IN/83/AG83 was isolated in 1985 from a human with visceral leishmaniasis in West Bengal, India. 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-50602 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: 70030921 Manufacturing Date: 15NOV2019

TEST	SPECIFICATIONS	RESULTS
Cell Morphology¹ 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	Elongated, motile and refractile; rosettes visible
Genotypic Analysis ² Sequencing of internal transcribed spacer (ITS) 1, 5.8S ribosomal RNA gene, ITS 2 (~ 1050 base pairs) Sequencing of N-acetylglucosamine-1-phosphate transferase gene (nagt) (~ 1320 base pairs)	≥ 99% sequence identity to L. donovani, strain MHOM/IN/00/DEVI (GenBank: AJ634376.1) ≥ 99% sequence identity to L. donovani nagt gene (GenBank: DQ836150.1)	99.9% sequence identity to L. donovani, strain MHOM/IN/00/DEVI (GenBank: AJ634376.1) ³ 100% sequence identity to L. donovani nagt gene (GenBank: DQ836150.1) ⁴
Viable Cell Count by Hemacytometry ²	> 10 ⁶ cells per mL	1.4 × 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 ⁵ Trypticase soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic	No growth No growth No growth	No growth No growth No growth
DMEM with 10% FBS, 37°C, aerobic Sheep blood agar, 37°C, aerobic Sheep blood agar, 37°C, anaerobic	No growth No growth No growth	No growth No growth No growth
Thioglycollate broth, 37°C, anaerobic Tooting completed on violed, next freeze meterial	No growth	No growth

¹Testing completed on vialed, post-freeze material

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²Testing completed on bulk material prior to vialing and freezing

³Also consistent with *L. infantum*, a member 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.].

⁴L. donovani is differentiated from L. infantum based on a 2-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." <u>Eukaryot. Cell</u> 6 (2007): 198-210. PubMed: 17142569.].

⁵Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.



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/Heather Couch/

Heather Couch 10 JUN 2020

Program Manager or designee, ATCC Federal Solutions

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