

Certificate of Analysis for NR-50181

Leishmania major, Strain LV39 Clone 5 (+luc)

Catalog No. NR-50181

Product Description:

Leishmania major (L. major), strain LV39c5 (+luc) is a transgenic clone derived from strain LV39 (MRHO/SU/59/P), which was originally isolated in 1959 from a gerbil in southern Russia. NR-50181 lot 61233613 was produced by cultivation of the deposited material in Modified Medium 199 (M199) with Hanks' salts supplemented with 10% heat-inactivated fetal bovine serum (HIFBS) 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: 64233613 Manufacturing Date: 06MAY2016

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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 (~ 1120 base pairs)	≥ 99% sequence identity to L. major, strain LV39c5 (GenBank: AODR01000399.1)	100% sequence identity to L. major, strain LV39c5 (GenBank: AODR01000399.1)
Presence of Luciferase Gene by PCR Amplification ²	~ 1600 base pair amplicon	~ 1600 base pair amplicon
Functional Activity of Luciferase Gene ^{2,3}	Positive	Positive
Viable Cell Count by Hemacytometry ²	> 10 ⁶ cells per mL	3.4 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, aerobic⁴	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

¹Testing completed on vialed, post-freeze material

/Heather Couch/

Heather Couch 16 JUL 2020

Program Manager or designee, ATCC Federal Solutions

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²Testing completed on bulk material prior to vialing and freezing

³Luciferase 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.]

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