

Certificate of Analysis for NR-51210

Leishmania mexicana, Strain MNYC/BZ/62/M379 (+luc)

Catalog No. NR-51210

Product Description:

Leishmania mexicana (L. mexicana), strain MNYC/BZ/62/M379 (+luc) is a transgenic clone derived from the parent MNYC/BZ/62/M379 strain. Strain MNYC/BZ/62/M379, also referred to as M 379 or MNYZ/BZ/62/M379, was originally isolated in 1962 from a *Nyctomys sumichrasti* (vesper rat) in Cayo District, Belize (formerly British Honduras). NR-51210 lot 70014487 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 3 days at 25°C in an aerobic atmosphere to produce this lot.

Lot: 70014487 Manufacturing Date: 05APR2018

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	Elongated, motile, refractile
Genotypic Analysis ² Sequencing of internal transcribed spacer (ITS) 1, 5.8S ribosomal RNA gene, ITS 2 (~ 1010 base pairs)	≥ 99% sequence identity to <i>L. mexicana</i> , strain MNYC/BZ/62/M379 (GenBank: AF466383.1)	99.0% sequence identity to <i>L. mexicana</i> , strain MNYC/BZ/62/M379 (GenBank: AF466383.1)
Functional Activity of Luciferase Gene ^{2,3}	Positive	Positive
Viable Cell Count by Hemacytometry ²	> 10 ⁶ cells per mL	5.4 × 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 ⁴ 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	No growth No growth	No growth No growth
Sheep blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic	No growth No growth	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.

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