b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

# Certificate of Analysis for NR-21979

## *Leishmania mexicana*, Strain Δ*lmgt*

## Catalog No. NR-21979

**Product Description:** *Leishmania mexicana (L. mexicana)*, strain Δ*lmgt* was constructed by targeted gene replacement of the three *Leishmania* glucose transporter (*LmGT*) alleles of strain MNYC/BZ/62/M379 with puromycin acetyltransferase and streptothricin acetyltransferase genes (encoding resistance markers for the antibiotics puromycin and nourseothricin, respectively). Strain MNYC/BZ/62/M379 was originally isolated from *Nyctomys sumichrasti* (rat) in Cayo District, Belize, 1962.

**Lot[1]: 60704412** **Manufacturing Date: 02APR2012**

| TEST | SPECIFICATIONS | RESULTS |
|---|---|---|
| **Genotyping** | | |
| Sequencing of 18S ribosomal RNA gene and internal transcribed spacer (ITS) 1 (~ 1010 base pairs) | Consistent with *Leishmania* species | Consistent with *Leishmania* species |
| Sequencing of ITS 1, 5.8S ribosomal RNA gene, ITS 2 (~ 950 base pairs) | Consistent with *L. mexicana* species complex[2] | Consistent with *L. mexicana* species complex[2,3] |
| **PCR Assay of Extracted DNA** | | |
| 18S ribosomal RNA gene | ~ 2500 base pair amplicon | ~ 2500 base pair amplicon |
| ITS 1, 5.8S ribosomal RNA gene, ITS 2 | ~ 1200 base pair amplicon | ~ 1200 base pair amplicon |
| **Confirmation of Δ*LmGT1* by PCR Assay of Extracted DNA** | | |
| *L. venezuelensis*, strain MHOM/VE/80/H-16 (NR-29184)[2] | ~ 2500 base pair amplicon | ~ 2500 base pair amplicon |
| *L. mexicana*, strain Δ*lmgt* (NR-21979) | No amplicon | No amplicon |
| **Viable Cell Count by Hemacytometry (pre-freeze)** | > $10^6$ cells/mL | 4.9 x $10^7$ cells/mL |
| **Viability (post-freeze)[4]** | Growth | Growth |
| **Sterility (21-day incubation)** | | |
| Harpo's HTYE broth[5], 37°C and 26°C, aerobic | 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 |
| Brain heart infusion, 37°C and 26°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]NR-21979 was produced by cultivation of the deposited material in Modified M199 medium supplemented with 10% heat-inactivated fetal bovine serum and 10 µg/mL hemin for 5 days at 25°C aerobic atmosphere to produce this lot.
[2]*L. mexicana* species complex (*L. mexicana*, *L. amazonensis* and *L. venezuelensis*)
[3]>99% identical to *L. mexicana,* strain MNYC/BZ/62/M379 (GenBank: AF466383.1)
[4]Viable cells were observed after 4 days under cultivation conditions.
[5]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

**Date:** 09 AUG 2013 **Signature:**

**Title:** Technical Manager, BEI Authentication or designee

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