

Certificate of Analysis for NR-49247

Leishmania amazonensis, Strain LV78 (+bla)

Catalog No. NR-49247

Product Description: Leishmania amazonensis (L. amazonensis), strain LV78 (+bla) is a transgenic clone derived from strain LV78 (RAT/BR/72/LV78), which was originally isolated in 1972 from a rat with cutaneous leishmaniasis in Brazil.

Lot¹: 63769236 Manufacturing Date: 30SEP2015

TEST	SPECIFICATIONS	RESULTS
Genotyping Sequencing of internal transcribed spacer (ITS) 1, 5.8S ribosomal RNA gene, ITS 2 (~ 670 base pairs)	Consistent with L. amazonensis	Consistent with <i>L. amazonensis</i> ²
Functional Activity by PCR Amplification ITS 1, 5.8S ribosomal RNA gene, ITS 2	900 to 1200 base pair amplicon	~ 1100 base pair amplicon
Phenotypic Analysis β-lactamase activity³	Positive	Positive
Viable Cell Count by Hemacytometry (pre-freeze)	> 10 ⁶ cells/mL	9 × 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 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

¹NR-49247 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 4 days at 25°C in an aerobic atmosphere to produce this lot.

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Date: 08 JUN 2016 Signature:

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²Also consistent with other *Leishmania* species

³Parasites were lysed in 0.1% Nonidet P-40 and incubated with nitrocephin substrate for 30 minutes at 37°C. A change in color from yellow to red indicates the presence of β-lactamase activity (Buckner, F. S., et al. "Colorimetric Assay for Screening Compounds against *Leishmania* Amastigotes Grown in Macrophages." Am. J. Trop. Med. Hyg. 72 (2005): 600-605. PubMed: 15891135.).

⁴Viable cells were observed after 1 day in Modified M199 medium supplemented with 10% heat-inactivated fetal bovine serum and 10 μg/mL hemin at 25°C in an aerobic atmosphere.

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