

# **Certificate of Analysis for NR-50176**

## Leishmania tropica, Strain LRC-L590 (+luc)

## Catalog No. NR-50176

### **Product Description:**

Leishmania tropica (L. tropica), strain LRC-L590 (+luc) is a transgenic clone derived from strain LRC-L590 (MHOM/IL/1990/P283). Strain LRC-L590 (MHOM/IL/1990/P283), also referred to as MHOM/IL/1990/LRC-590 and L590, was originally isolated in 1990 from an 11-year-old male with cutaneous leishmaniasis in Kfar Adumim, Judean Desert, Israel. NR-50176 lot 70002272 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  $\mu$ g/mL hemin for 4 days at 25°C in an aerobic atmosphere to produce this lot.

Lot: 70002272 Manufacturing Date: 07FEB2017

TEST	SPECIFICATIONS	RESULTS
	3FEGII ICATIONS	RESOLIS
Cell Morphology <sup>1</sup> 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 <sup>2</sup> Sequencing of internal transcribed spacer (ITS) 1, 5.8S ribosomal RNA gene, ITS 2 (~ 910 base pairs)	Consistent with L. tropica	Consistent with L. tropica
Functional Activity by PCR Amplification <sup>3</sup> ITS 1, 5.8S rRNA gene, ITS 2	~ 1200 base pair amplicon	~ 1200 base pair amplicon
Functional Activity of Luciferase Gene <sup>2,3</sup>	Positive	Positive
Viable Cell Count by Hemacytometry <sup>2</sup>	> 10 <sup>6</sup> cells per mL	3.48 x 10 <sup>8</sup> cells/mL
Viability <sup>1</sup> 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) <sup>1</sup>		
Harpo's HTYE broth, 37°C and 26°C, aerobic <sup>4</sup> Trypticase soy broth, 37°C and 26°C, aerobic	No growth No growth	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

<sup>&</sup>lt;sup>1</sup>Testing completed on vialed, post-freeze material

### /Heather Couch/

Heather Couch 16 JUL 2020

Program Manager or designee, ATCC Federal Solutions

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BEI Resources www.beiresources.org E-mail: <a href="mailto:contact@beiresources.org">contact@beiresources.org</a>
Tel: 800-359-7370

Fax: 703-365-2898

<sup>&</sup>lt;sup>2</sup>Testing completed on bulk material prior to vialing and freezing

<sup>&</sup>lt;sup>3</sup>Luciferase 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.]

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