

Certificate of Analysis for NR-40347

Trypanosoma cruzi, Strain Brazil (+luc)

Catalog No. NR-40347

Product Description:

Trypanosoma cruzi (T. cruzi), strain Brazil (+luc) is a transgenic clone derived from the Brazil strain, which was isolated from a human patient in Brazil. Strain Brazil (+luc) was deposited to BEI Resources as a luciferase-expressing strain in the trypomastigote stage of the parasite's life cycle. Luciferase was introduced via the pTREX-GFP plasmid where luc replaced GFP. NR-40347 was produced by cultivation of BEI Resources seed lot 61447873 in BS-C-1 monkey kidney epithelial cells (ATCC® CCL-26™) with Eagle's Minimum Essential Medium (EMEM) supplemented with 10% heat-inactivated fetal bovine serum (HIFBS) for 6 days at 37°C in an aerobic atmosphere with 5% CO₂ until emergence of trypomastigotes from host cells was observed to produce this lot.

Lot: 70038190 Manufacturing Date: 08SEP2020

TEST	SPECIFICATIONS	RESULTS
Cell Morphology ¹ 3 days at 37°C in an aerobic atmosphere with 5% CO ₂ in EMEM supplemented with 10% HIFBS	Report results	Elongated and refractile
Genotypic Analysis ² Sequencing of 18S ribosomal RNA gene (~ 800 base pairs) Sequencing of putative C-5 sterol desaturase gene (TcSC5D) (~ 730 base pairs)	Consistent with <i>T. cruzi</i> ≥ 99% sequence identity to <i>T. cruzi</i> Discrete Typing Unit (DTU) <i>T. cruzi</i> I (TcI) (GenBank: JN050567.1)	Consistent with <i>T. cruzi</i> 99.2% sequence identity to <i>T. cruzi</i> DTU Tcl (GenBank: JN050567.1) ³
Functional Activity of Luciferase Gene ^{1,4}	Positive	Positive
Viable Cell Count by Hemacytometry ^{2,5}	> 10 ⁶ cells per mL	1.2 × 10 ⁷ cells per mL
Viability ¹ 3 days at 37°C in an aerobic atmosphere with 5% CO ₂ in EMEM supplemented with 10% HIFBS	Growth	Growth
Sterility (21-day incubation) ¹ Harpo's HTYE broth, 37°C and 26°C, aerobic ⁶ 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 Mycoplasma Contamination ¹	No growth	No growth
DNA Detection by PCR	None detected	None detected

¹Testing completed on vialed, post-freeze material.

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²Testing completed on bulk material prior to vialing and freezing.

³Cosentino, R. O. and F. Agüero. "A Simple Strain Typing Assay for *Trypanosoma cruzi*: Discrimination of Major Evolutionary Lineages from a Single Amplification Product." PLoS Negl. Trop. Dis. 6 (2012): e1777. PubMed: 22860154.

⁴Luciferase activity was determined using the Luciferase Assay System (Promega E1500). Parasites were lysed and incubated with luciferase assay reagent. Luciferase activity was measured using a luminometer with a bioluminescence emission spectra of ~ 560 nm.

⁵Only trypomastigote-stage parasites were counted.

⁶Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.



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/Heather Couch/

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Program Manager or designee, ATCC Federal Solutions

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