

Certificate of Analysis for NR-18959

Trypanosoma cruzi, Strain Tulahuen, Clone C4 (+lacZ)

Catalog No. NR-18959

Product Description: *Trypanosoma cruzi* (*T. cruzi*), strain Tulahuen, clone C4 (+*lacZ*) is a transgenic clone derived from the Tulahuen strain.

Lot¹: 59490913 Manufacturing Date: 24SEP2010

TEST	SPECIFICATIONS	RESULTS
Genotyping Sequencing of 18S rRNA gene (~ 1090 bp)	Consistent with <i>T. cruzi</i>	Consistent with <i>T. cruzi</i>
Phenotypic Analysis ² NR-18959 β-Galactosidase activity Negative control (ATCC [®] CCL-163™)	Positive (red) Negative (yellow)	Positive (red) Negative (yellow)
Viable Cell Count by Hemacytometry (pre-freeze) ³	> 10 ⁶ cells/mL	4 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 Trypticase soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic Brain heart infusion, 37°C and 26°C, aerobic Sheep blood agar, 37°C, aerobic Sheep blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic	No growth	No growth
Mycoplasma Contamination DNA Detection by PCR	None detected	None detected

¹NR-18959 was produced by cultivation of the deposited material BALB/3T3 clone A31 mouse embryonic fibroblasts (ATCC[®] CCL-163™) with cell cultivation medium for parasites (ATCC medium 2222: adjusted to contain 10% heat-inactivated fetal bovine serum). The culture was propagated in 95% air, 5% CO₂ for 4 days at 37°C, until emergence of trypomastigotes from host cells was observed.

Date: 05 APR 2011

Signature:

Title:

Technical Manager, BEI Authentication or designee

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²Parasites (~ 10⁷) were lysed in 0.1% Triton X-100 and PBS and 100 μM chlorophenol red-β-D-galactoside (CPRG) and incubated for 5 min. at 37°C [Buckner, F. S., et al. "Efficient Technique for Screening Drugs for Activity against *Trypanosoma cruzi* using Parasites Expressing β-Galactosidase." Antimicrob. Agents Chemother. 40 (1996): 2592-2597. PubMed: 8913471.].

³Only trypomastigote-stage parasites were counted

⁴Viable cells and signs of infection were seen after 3 days under cultivation conditions at 37°C

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