

Certificate of Analysis for NR-18961

Trypanosoma cruzi, Strain Sonya, Clone B10 (+lacZ)

Catalog No. NR-18961

Product Description: *Trypanosoma cruzi* (*T. cruzi*), strain Sonya, clone B10 (+*lacZ*) is a transgenic clone derived from the Sonya strain.

Lot¹: 59490919 Manufacturing Date: 10NOV2010

TEST	SPECIFICATIONS	RESULTS
Genotyping Sequencing of 18S rRNA gene (~ 960 bp)	Consistent with <i>T. cruzi</i>	Consistent with <i>T. cruzi</i>
Phenotypic Analysis ² NR-18961 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	2.7 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
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¹NR-18961 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 6 days at 37°C, until emergence of trypomastigotes from host cells was observed.

Date: 06 APR 2011

Signature:

Title: Technical Manager, BEI Authentication or designee

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

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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 20 min. at 37°C [Buckner, F. S., et al. "Efficient Technique for Screening Drugs for Activity against *Trypanosoma cruzi* using Parasites Expressing β-Galactosidase." <u>Antimicrob. Agents Chemother.</u> 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. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.