

***Trypanosoma cruzi*, Strain CL Brener (+*luc*)**

Catalog No. NR-49161

Product Description:

Trypanosoma cruzi (*T. cruzi*), strain CL Brener (+*luc*) is a transgenic clone derived from strain CL Brener. Strain CL Brener is a clone of strain CL, a genotype *T. cruzi* VI (TcVI) strain isolated in 1963 from naturally infected *Triatoma infestans* (kissing bug) collected from a house in Rio Grande do Sul, Brazil. NR-49161 lot 70015225 was produced by cultivation of the deposited material in Liver Infusion Tryptose (LIT) medium supplemented with 10% heat-inactivated fetal bovine serum (HIFBS) and 7.5 µg/mL hemin for 3 days at 25°C in an aerobic atmosphere.

Lot: 70015225

Manufacturing Date: 30APR2018

TEST	SPECIFICATIONS	RESULTS
Genotypic Analysis¹ Sequencing of putative C-5 sterol desaturase gene (<i>TcSC5D</i>) (~ 750 base pairs) Sequencing of <i>Ppy RE9</i> gene (760 base pairs) ³	≥ 99% sequence identity to <i>T. cruzi</i> Discrete Typing Unit (DTU) ≥ 99% sequence identity to <i>Ppy RE9</i> (GenBank: GQ404466.1)	99.3% sequence identity to <i>T. cruzi</i> DTU TcVI ² 100% sequence identity to <i>Ppy RE9</i> (GenBank: GQ404466.1)
Functional Activity by PCR Amplification⁴ <i>TcSC5D</i> ⁵ <i>Ppy RE9</i> ⁶	~ 830 base pair amplicon ~ 830 base pair amplicon	~ 830 base pair amplicon ~ 830 base pair amplicon
Functional Activity of Luciferase Gene^{4,7}	Positive	Positive
Viable Cell Count by Hemacytometry⁴	> 10 ⁶ cells per mL	1.6 × 10 ⁸ cells per mL
Viability¹ 10 days at 25°C in an aerobic atmosphere in LIT medium supplemented with 10% HIFBS and 7.5 µg/mL hemin	Growth	Growth
Sterility (21-day incubation)¹ Harpo's HTYE broth, 37°C and 26°C, aerobic ⁸ 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 No growth No growth No growth No growth No growth	No growth No growth No growth No growth No growth No growth No growth

¹Testing completed on vial, post-freeze material.

²Also consistent with genotype TcV. Genotypes TcV and TcVI cannot be distinguished [Cosentino, 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.].

³*Ppy RE9*; thermostable, red-shifted *Photinus pyralis* (North American firefly) luciferase gene mutant [Branchini, B. R., et al. "Red-Emitting Luciferases for Bioluminescence Reporter and Imaging Applications." *Anal. Biochem.* 396 (2010): 290-297. PubMed: 19748472.]

⁴Testing completed on bulk material prior to vialing and freezing.

⁵PCR was performed as described in Cosentino, 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.

⁶Primers and PCR conditions are available upon request.

⁷Luciferase 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 ~ 612 nm.

⁸Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

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16 JUL 2020

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