

Certificate of Analysis for NR-46428

Trypanosoma cruzi, Strain TcVT-1 (axenic epimastigote)

Catalog No. NR-46428

Product Description:

Trypanosoma cruzi (T. cruzi), strain TcVT-1 was isolated from the blood of a five-year-old female English Cocker Spaniel with Chagas' disease in October 2012 in Lynchburg, Virginia, USA. T. cruzi, strain TcVT-1 was deposited as the trypomastigote form of the parasite's life cycle and a genotype TcIV strain. NR-46428 consists of the epimastigote stage, which was established by BEI Resources from the trypomastigote form over multiple passages in culture.

Lot: 70022534¹ Manufacturing Date: 29JAN2019

TEST	SPECIFICATIONS	RESULTS
Cellular Morphology ²	Report results	Elongated, motile cells and rosette structures
Genotypic Analysis³ Sequencing of 18S ribosomal RNA (rRNA) gene (1530 base pairs)	Consistent with <i>T. cruzi</i>	Consistent with <i>T. cruzi</i> ⁴
Sequencing of putative C-5 sterol desaturase gene (<i>TcSC5D</i>) (~ 800 base pairs)	≥ 99% sequence identity to T. cruzi Discrete Typing Unit (DTU) T. cruzi (TcIV)	99.9% sequence identity to T. cruzi Discrete Typing Unit (DTU) T. cruzi (TcIV)
Functional Activity by PCR Amplification ³ 18S ribosomal RNA gene ⁵	~ 2200 base pair amplicon	~ 2500 base pair amplicon
TcSC5D ⁶	~ 830 base pair amplicon	~ 830 base pair amplicon
Viable Cell Count by Hemacytometry ³	> 10 ⁶ cells/mL	2.0 × 10 ⁷ cells/mL
Viability (post-freeze) ^{2,7}	Growth	Growth
Sterility (21-day incubation) ²		
Harpo's HTYE broth, 37°C and 26°C, aerobic ⁸	No growth	No growth
Trypticase soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud broth, 37°C and 26°C, aerobic	No growth	No growth
Brain heart infusion, 37°C and 26°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

¹NR-46428 was produced by cultivation of BEI Resources NRS-46428 lot 62415819 in Liver Infusion Tryptose (LIT) medium (ATCC[®] medium 1029) supplemented with 10% heat-inactivated fetal bovine serum (HIFBS) and 1% hemin for 4 days at 25°C in an aerobic atmosphere to produce this lot.

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²Testing completed on vialed, post-freeze material

³Testing completed on bulk material prior to vialing and freezing

⁴Also consistent with other *Trypanosoma* species

⁵Primer sequences and conditions for PCR are available upon request.

⁶PCR 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." <u>PLoS Negl. Trop. Dis.</u> 6 (2012): e1777. PubMed: 22860154.

Viable cells and signs of infection were seen after 1 day at 25°C in an aerobic atmosphere in LIT medium supplemented with 10% HIFBS and 1% hemin.

⁸Atlas, 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/ Heather Couch

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Program Manager or designee, ATCC Federal Solutions

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