

Certificate of Analysis for NR-48851

Trypanosoma brucei subsp. brucei, Strain TREU 667 (in vitro procyclic form)

Catalog No. NR-48851

Product Description: Trypanosoma brucei (T. brucei) subsp. brucei, strain TREU 667 (in vitro procyclic form) was harvested from the blood of infected BALB/c mice and adapted to cell culture by BEI Resources. The parent strain TREU 667 (available as BEI Resources NR-46440) was originally isolated in Kenya. The TREU 667 (Trypanosomiasis Research Edinburgh University) strain is a pleomorphic strain of low virulence to mice.

Lot¹: 62817460 Manufacturing Date: 24JUL2014

TEST	SPECIFICATIONS	RESULTS
Genotyping Sequencing of internal transcribed spacer (ITS) 1, 5.8S ribosomal RNA gene, ITS 2 (~ 460 base pairs)	Consistent with <i>T. brucei</i>	Consistent with <i>T. bruce</i> ²
Functional Activity by PCR Amplification ITS 1, 5.8S ribosomal RNA gene, ITS 2 ³	~ 1300 base pair amplicon	~ 1300 base pair amplicon
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

NR-48851 was produced by adaptation of *T. brucei* subsp. *brucei*, strain TREU 667 to *in vitro* growth in Trypanosome medium (ATCC[®] Medium 431) at 27°C in an aerobic atmosphere, followed by subculture in SDM-79 medium (Life Technologies, custom order part number ME090164 P1) adjusted to contain 10% (v/v) heat-inactivated fetal bovine serum (HIFBS) and 7.5 µg/mL hemin. The culture was propagated for 3 days at 27°C in an aerobic atmosphere.

Date: 27 FEB 2015 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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BEI Resources

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²Also consistent with \dot{T} . evansi and/or T. equiperdum, which are putative subspecies of T. brucei (Lun, Z. R., et al. "Trypanosoma brucei: Two Steps to Spread Out from Africa." Trends Parasitol. 26 (2010): 424-427. PubMed: 20561822.)

³PCR was performed as described in Agbo, E. C., et al. "Measure of Molecular Diversity within the *Trypanosoma brucei* Subspecies *Trypanosoma* brucei brucei and Trypanosoma brucei gambiense as Revealed by Genotypic Characterization." Exp. Parasitol. 99 (2001): 123-131. PubMed: 11846522.

⁴Viable cells were seen after 5 days under cultivation conditions.

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