

Certificate of Analysis for NR-44388

Trypanosoma brucei subsp. brucei, Strain STIB 247 (in vitro)

Catalog No. NR-44388

Product Description: *Trypanosoma brucei* (*T. brucei*) subsp. *brucei*, strain STIB 247 (*in vitro*) was harvested from the blood of infected BALB/c mice and adapted to cell culture by BEI Resources. The parent strain STIB 247 (BEI Resources NR-36197) was isolated in 1971 from a hartebeest in Serengeti National Park, Serengeti, Tanzania, and prior to freezing, was passaged in rats for an unknown number of days.

Lot¹: 62069849 Manufacturing Date: 24SEP2013

TEST	SPECIFICATIONS	RESULTS
Genotyping		
Sequencing of 18S ribosomal RNA (rRNA) gene (~ 1520 base pairs)	Consistent with <i>T. brucei</i>	Consistent with <i>T. bruce</i> ²
Sequencing of internal transcribed spacer (ITS) 1, 5.8S rRNA gene, ITS 2 (~ 400 base pairs)	Consistent with <i>T. brucei</i>	Consistent with <i>T. brucel</i> ²
Functional Activity by PCR Amplification		
18S rRNA gene ³	~ 2200 base pair amplicon	~ 2200 base pair amplicon
ITS 1, 5.8S rRNA gene, ITS 2 ⁴	~ 1300 base pair amplicon	~ 1300 base pair amplicon
Viable Cell Count by Hemacytometry (pre-freeze)	> 10 ⁶ cells/mL	3 x 10 ⁷ cells/mL
Viability (post-freeze) ⁵	Growth	Growth
Sterility (21-day incubation)		
Harpo's HTYE broth ⁶ , 37°C and 26°C, aerobic	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-44388 was produced by adaptation of *T. brucei* subsp. *brucei*, strain STIB 247 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 4 days at 27°C in an aerobic atmosphere.

Date: 24 JUL 2014 Signature:

Title: Technical Manager, BEI Authentication or designee

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²Also consistent with *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." <u>Trends Parasitol</u>. 26 (2010): 424-427. PubMed: 20561822.)

³Primer sequences and conditions for PCR are available upon request.

⁴PCR 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." <u>Exp Parasitol.</u> 99 (2001): 123-131. PubMed: 11846522.

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

⁶Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.