Certificate of Analysis for NR-44389

Trypanosoma brucei subsp. *gambiense*, Strain STIB 386 *(in vitro)*

Catalog No. NR-44389

Product Description:

Trypanosoma brucei (T. brucei) subsp. *brucei*, strain STIB 386 *(in vitro)* was harvested from the blood of infected BALB/c mice and adapted to cell culture by BEI Resources. The parent strain STIB 386 (BEI Resources NR-36198) was derived from strain TH 114/78E (020), which was isolated in 1978 from a male patient in Koudougou, Ivory Coast, West Africa. NR-44389 lot 70022602 was produced by cultivation of BEI Resources seed lot 62069847 in SDM-79 medium (Life Technologies, custom order part number ME090164 P1) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (HIFBS) and 7.5 µg/mL hemin for 5 days at 27°C in an aerobic atmosphere.

Lot: 70022602 **Manufacturing Date: 30JAN2019**

TEST	SPECIFICATIONS	RESULTS
Cellular Morphology[1] 1 day at 27°C in an aerobic atmosphere in SDM-79 medium supplemented with 10% HIFBS and 7.5 µg/mL hemin	Report results	Elongated and refractive; rosettes visible
Genotypic Analysis[2] Sequencing of internal transcribed spacer (ITS) 1, 5.8S ribosomal (rRNA) gene, ITS 2 (~ 1090 base pairs)	≥ 98% sequence identity to *T. brucei* subsp. *gambiense*, strain DA1972 (GenBank: AF306774.1)	98.2% sequence identity to *T. brucei* subsp. *gambiense*, strain DA1972 (GenBank: AF306774.1)[3]
PCR Assay of Extracted DNA[2] Serum resistance-associated gene (SRA)[4]	No amplicon	No amplicon
Viable Cell Count by Hemacytometry[3]	> 10^6 cells/mL	1 × 10^8 parasites/mL
Viability[2] 1 day at 27°C in an aerobic atmosphere in SDM-79 medium supplemented with 10% HIFBS and 7.5 µg/mL hemin	Growth	Growth
Sterility (21-day incubation)[2]		
Harpo's HTYE broth[5], 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
DMEM with 10% FBS, 37°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

[1]Testing completed on vialed, post-freeze material.
[2]Testing completed on bulk material prior to vialing and freezing.
[3]Also 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." Trends Parasitol. 26 (2010): 424-427. PubMed: 20561822.)
[4]*T. brucei* subsp. *gambiense* is differentiated from *T. brucei* subsp. *rhodesiense* by lack of the SRA gene. (Radwanska, M., et al. "The Serum Resistance-Associated Gene as a Diagnostic Tool for the Detection of *Trypanosoma brucei rhodesiense*." Am. J. Trop. Med. Hyg. 67 (2002): 684-690. PubMed: 12518862.).
[5]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

/Heather Couch/
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