

Certificate of Analysis for NR-53513

Trypanosoma brucei subsp. gambiense, Strain 348BT

Catalog No. NR-53513

Product Description:

Trypanosoma brucei (*T. brucei*) subsp. *gambiense*, strain 348BT was isolated in 2006 from the cerebral spinal fluid of a human with African trypanosomiasis (sleeping sickness) before treatment in Mbuji-Mayi, Democratic Republic of Congo. NR-53513 was produced by cultivation of the deposited material in modified HMI-9 medium supplemented with 10% heat-inactivated fetal bovine serum (HIFBS) for 1 day at 37°C in an aerobic atmosphere with 5% CO₂ to produce this lot.

Lot: 70038192 Manufacturing Date: 14OCT2020

TEST	SPECIFICATIONS	RESULTS
Cell Morphology ¹ 2 days at 37°C in an aerobic atmosphere with 5% CO ₂ in modified HMI-9 medium supplemented with 10% HIFBS	Report results	Elongated and motile, rosettes visible
Genotypic Analysis ² Sequencing of 18S ribosomal RNA gene (~ 890 base pairs) Sequencing of internal transcribed spacer (ITS) 1, 5.8S rRNA gene, ITS 2 (~ 1070 base pairs)	≥ 99% sequence identity to T. brucei subsp. gambiense, strain TgB1 (GenBank: KX007996.1) ≥ 98% sequence identity to T. brucei	99.7% sequence identity to T. brucei subsp. gambiense, strain TgB1 (GenBank: KX007996.1) ³ 99.3% sequence identity to T. brucei
Viable Cell Count by Hemacytometry ²	> 10 ⁶ cells per mL	1.6 × 10 ⁷ cells per mL
Viability ¹ 2 days at 37°C in an aerobic atmosphere with 5% CO ₂ in modified HMI-9 medium supplemented with 10% HIFBS	Growth	Growth
Sterility (21-day incubation) ¹ Harpo's HTYE broth, 37°C and 26°C, aerobic ⁴ 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	No growth No growth No growth No growth No growth No growth	No growth No growth No growth No growth No growth No growth
Thioglycollate broth, 37°C, anaerobic	No growth	No growth

¹Testing completed on vialed, post-freeze material.

/Heather Couch/

Heather Couch 16 JUL 2021

Program Manager or designee, ATCC Federal Solutions

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²Testing completed on bulk material prior to vialing and freezing.

³Also 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.].

⁴Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.