

Certificate of Analysis for NR-41946

Trypanosoma brucei subsp. brucei, Strain TREU 927/4 (GUTat 10.1 Clone)

Catalog No. NR-41946

Product Description: *Trypanosoma brucei* (*T. brucei*) subsp. *brucei*, strain TREU 927/4 (GUTat 10.1 clone) was derived from isolate GPAL/KE/70/EATRO 1534, which was obtained in 1970 from wild-caught *Glossina pallidipes* (tsetse flies) in Kiboko, Kenya. The isolate was passaged 12 times in mice to generate the TREU 927/4 strain, which was subjected to 27 rapid syringe passages in mice to produce the GUTat 10.1 clone.

Lot¹: 61730299 Manufacturing Date: 13MAY2013

TEST	SPECIFICATIONS	RESULTS
Genotyping Sequencing of 18S ribosomal RNA gene (~ 1580 base pairs) Sequencing of internal transcribed spacer (ITS) 1, 5.8S ribosomal RNA gene, ITS 2 (~ 1000 base pairs)	Consistent with <i>T. brucei</i> Consistent with <i>T. brucei</i>	Consistent with <i>T. brucei</i> ² Consistent with <i>T. brucei</i> ²
Functional Activity by PCR Amplification 18S ribosomal RNA gene ITS 1, 5.8S ribosomal RNA gene, ITS 2 ³	~ 2200 base pair amplicon ~ 1300 base pair amplicon	~ 2200 base pair amplicon ~ 1300 base pair amplicon
Viable Cell Count by Hemacytometry (pre-freeze)	> 10 ⁶ cells/mL	4.5 x 10 ⁶ cells/mL
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
Mycoplasma Contamination DNA Detection by PCR	None detected	None detected

¹NR-41946 was produced by cultivation of the deposited material 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 ambient atmosphere.

Date: 21 NOV 2013

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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.)

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 4 days under cultivation conditions.

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