

Certificate of Analysis for NR-36198

Trypanosoma brucei subsp. gambiense, Strain STIB 386

Catalog No. NR-36198

Product Description: *Trypanosoma brucei* (*T. brucei*) subsp. *gambiense*, strain STIB 386 was derived from strain TH 114/78E (020), which was isolated in 1978 from a male patient in Koudougou, Ivory Coast, West Africa.

Lot¹: 61825547 Manufacturing Date: 10JUN2013

TEST	SPECIFICATIONS	RESULTS
Genotyping Sequencing of 18S ribosomal RNA gene (~ 1660 base pairs) Sequencing of internal transcribed spacer (ITS) 1, 5.8S ribosomal RNA gene, ITS 2 (~ 290 base pairs)	Consistent with <i>T. brucei</i> Consistent with <i>T. brucei</i>	Consistent with <i>T. brucei</i> Consistent with <i>T. brucei</i>
Functional Activity by PCR Amplification 18S ribosomal RNA gene ³ ITS 1, 5.8S ribosomal RNA gene, ITS 2 ⁴	~ 2200 base pair amplicon ~ 1300 base pair amplicon	~ 2200 base pair amplicon ~ 1300 base pair amplicon
Level of Parasitemia (pre-freeze) ⁵	≥ 1 x 10 ⁷ parasites/mL	8.7 x 10 ⁷ parasites/mL
Viability (post-freeze) ⁶	Confirmed	Confirmed

NR-36198 was produced by inoculation of the deposited material into a Balb/c mouse. Infection was allowed to progress for 5 days until the first peak of parasitemia was reached. Infected blood was collected by orbital bleeding and used to inoculate ten Balb/c mice. Infection was allowed to progress for 5 days until the first peak of parasitemia was reached and infected blood was collected by orbital bleeding.

Date: 21 NOV 2013

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Signature:

Title:

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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.

⁵Parasitemia was determined after 5 days of infection by microscopic counts using a haemocytometer and 0.85% ammonium chloride as diluent.

⁶Viability of trypanosomes was confirmed by examination of Balb/c mouse for parasitemia at 7 days post infection.