

Certificate of Analysis for NR-46439

Trypanosoma brucei subsp. brucei, Strain Lab 110 EATRO

Catalog No. NR-46439

Product Description: *Trypanosoma brucei* (*T. brucei*) subsp. *brucei*, strain Lab 110 EATRO was previously obtained by the depositor from W. Trager, Rockefeller University, New York, New York, USA, where it has been maintained in culture at Pace University.

Lot¹: 62835190 Manufacturing Date: 11AUG2014

TEST	SPECIFICATIONS	RESULTS
Genotyping Sequencing of internal transcribed spacer (ITS) 1, 5.8S ribosomal RNA gene, ITS 2 (~ 480 base pairs)	Consistent with <i>T. brucei</i>	Consistent with <i>T. brucei</i> ²
Functional Activity by PCR Amplification ITS 1, 5.8S ribosomal RNA gene, ITS 2 ³	~ 1300 base pair amplicon	~ 1300 base pair amplicon
Level of Parasitemia (pre-freeze) ⁴	≥ 1 x 10 ⁶ parasites/mL	1.3 x 10 ⁸ parasites/mL
Viability (post-freeze) ⁵	Growth in inoculated mouse	Growth in inoculated mouse

¹NR-46439 was produced by inoculation of the deposited material into a BALB/c mouse. Infection was allowed to progress for 2 days until the first peak of parasitemia was reached. Infected blood was collected by orbital bleeding and used to inoculate six BALB/c mice. Infection was allowed to progress for 10 days until the first peak of parasitemia was reached and infected blood was collected by orbital bleeding.

Date: 09 NOV 2015 Signature:

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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.

⁴Parasitemia was determined after 10 days of infection by microscopic counts using a haemocytometer and 0.85% ammonium chloride as diluent.

⁵Viability of trypanosomes was confirmed by examination of BALB/c mice for parasitemia at 4 days post infection.