

Certificate of Analysis for NR-46433

Trypanosoma brucei subsp. rhodesiense, Strain KETRI 2285

Catalog No. NR-46433

Product Description: Trypanosoma brucei (T. brucei) subsp. rhodesiense, strain KETRI 2285 was isolated in 1976 from the blood of a patient before chemotherapy in Busoga, Uganda. T. brucei subsp. rhodesiense, strain KETRI 2285 was obtained by Professor C. J. Bacchi from the Kenya Trypanosomiasis Research Institute (KETRI) strain bank at Mugaga, Kenya.

Lot¹: 63717830 Manufacturing Date: 24AUG2015

TEST	SPECIFICATIONS	RESULTS
Genotyping Sequencing of internal transcribed spacer (ITS) 1, 5.8S ribosomal RNA gene, ITS 2 (~ 300 base pairs)	Consistent with <i>T. brucei</i>	Consistent with <i>T. brucef</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 per mL	2.2 × 10 ⁸ parasites per mL
Viability (post-freeze) ⁵	Growth in inoculated mouse	Growth in inoculated mouse

NR-46433 was produced by inoculation of the deposited material into a BALB/c mouse. Infection was allowed to progress for 3 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 4 days until the first peak of parasitemia was reached and infected blood was collected by orbital bleeding.

Date: 30 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:

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

⁵Viability of trypanosomes was confirmed by examination of a BALB/c mouse for parasitemia at daily intervals for 4 days post-infection.