

Certificate of Analysis for NR-49827

Trypanosoma brucei subsp. rhodesiense, Strain KETRI 243 (in vitro procyclic form)

Catalog No. NR-49827

Product Description: *Trypanosoma brucei* (*T. brucei*) subsp. *rhodesiense*, strain KETRI 243 (*in vitro* procyclic form) was harvested from the blood of infected BALB/c mice and adapted to cell culture by BEI Resources. Strain KETRI 243 (available as BEI Resources NR-46431, bloodstream form) was originally isolated in Busoga, Uganda, in 1961. The KETRI 243 (Kenya Trypanosomiasis Research Institute) strain has shown resistance to arsenical drugs, DL-α-difluoromethylornithine, diminazene, melarsen oxide, melarsoprol, pentamidine and suramin.

Lot^{1,2}: 63681646 Manufacturing Date: 04AUG2015

TEST	SPECIFICATIONS	RESULTS
Genotypic Analysis Sequencing of internal transcribed spacer (ITS) 1, 5.8S ribosomal RNA gene, ITS 2 (~ 290 base pairs) Sequencing of serum resistance-associated gene (SRA) (~ 560 base pairs)	Consistent with <i>T. brucei</i> Consistent with <i>T. brucei</i> subsp. rhodesiense	Consistent with <i>T. brucei</i> Consistent with <i>T. brucei</i> subsp. <i>rhodesiense</i> ⁴
PCR Assay of Extracted DNA ITS 1, 5.8S ribosomal RNA gene, ITS 2 ⁵ SRA ⁶	~ 1300 base pair amplicon ~ 610 base pair amplicon	~ 1300 base pair amplicon ~ 610 base pair amplicon
Viable Cell Count by Hemacytometry (pre-freeze)	> 10 ⁶ cells/mL	1 x 10 ⁸ cells/mL
Viability (post-freeze) ⁷	Growth	Growth
Sterility (21-day incubation) Harpo's HTYE broth ⁸ , 37°C and 26°C, aerobic 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 Thioglycollate broth, 37°C, anaerobic	No growth	No growth

NR-49827 was produced by adaptation of the deposited material from BALB/c mice to *in vitro* growth in Trypanosome medium (ATCC[®] Medium 431) at 27°C in an aerobic atmosphere, followed by subculture 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 over a period of several weeks. Once adapted, the culture was propagated for 4 days at 27°C in an aerobic atmosphere.

BEI Resources

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²Quality control testing completed on post-freeze material unless specified as pre-freeze.

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.)

⁴Radwanska, M., et al. "The Serum Resistance-Associated Gene as a Diagnostic Tool for the Detection of *Trypanosoma brucei rhodensiense*." <u>Am.</u> J. Trop. Med. Hyg. 67 (2002): 684-690. PubMed: 12518862.

⁵PCR 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.

⁶Primer sequences and conditions for PCR are available upon request.

⁷Viable cells were seen after 2 days under cultivation conditions.

⁸Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.



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Date: 21 APR 2016

Signature:

BEI Resources Authentication

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

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