

***Trypanosoma brucei* subsp. *rhodesiense*, Strain KETRI 2537 (in vitro procyclic form)**

Catalog No. NR-50075

Product Description: *Trypanosoma brucei* (*T. brucei*) subsp. *rhodesiense*, strain KETRI 2537 (in vitro procyclic form) was harvested from the blood of infected BALB/c mice and adapted to cell culture by BEI Resources. Strain KETRI 2537 (available as BEI Resources NR-46436, bloodstream form) was originally isolated in Busoga, Uganda, in 1972. The KETRI 2537 (Kenya Trypanosomiasis Research Institute) strain has demonstrated resistance to DL- α -difluoromethylornithine and susceptibility to diminazene aceturate when administered prior to the onset of a central nervous system infection in a mouse model.

Lot^{1,2}: 63901335

Manufacturing Date: 23NOV2015

TEST	SPECIFICATIONS	RESULTS
Genotypic Analysis Sequencing of internal transcribed spacer (ITS) 1, 5.8S ribosomal RNA gene, ITS 2 (~ 1300 base pairs) Sequencing of serum resistance-associated gene (SRA) (760 base pairs)	Consistent with <i>T. brucei</i> Consistent with <i>T. brucei</i> subsp. <i>rhodesiense</i>	Consistent with <i>T. brucei</i> ³ 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	2.3 × 10 ⁷ 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 No growth No growth No growth No growth No growth	No growth No growth No growth No growth No growth No growth No growth

¹NR-50075 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 5 days at 27°C in an aerobic atmosphere.

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." *Trends Parasitol.* 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 rhodesiense*." *Am. 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. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Date: 22 JUL 2016

Signature:



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