

Certificate of Analysis for NR-42010

Trypanosoma brucei subsp. brucei, Strain Lister 427 (procyclic form)

Catalog No. NR-42010

Product Description: *Trypanosoma brucei* (*T. brucei*) subsp. *brucei*, strain Lister 427 was deposited to BEI Resources in the procyclic form. Strain Lister 427 is a virulent lab strain that was isolated in 1960 from a sheep in Uganda and transferred to the Lister Institute in London in 1961. Strain Lister 427 is known to be a monomorphic, non-human-infective strain that is unable to complete development in the insect vector.

Lot¹: 61554575 Manufacturing Date: 15MAR2013

TEST	SPECIFICATIONS	RESULTS
Genotyping		
Sequencing of 18S ribosomal RNA gene (~ 1600 base pairs)	Consistent with T. brucei	Consistent with T. brucei
Sequencing of and internal transcribed spacer (ITS) 1, 5.8S ribosomal RNA gene, ITS 2 (~ 930 base pairs)	Consistent with <i>T. brucei</i>	Consistent with T. brucei
Functional Activity by PCR Amplification ² 18S ribosomal RNA gene ITS 1, 5.8S ribosomal RNA gene, ITS 2	~ 2200 base pair amplicon ~ 1300 base pair amplicons	~ 2200 base pair amplicon ~ 1300 base pair amplicons
Presence of T7 RNA Polymerase and Tetracycline Repressor (TetR/T7RNAP)	Negative	Negative
Viable Cell Count by Hemacytometry (pre-freeze)	> 10 ⁶ cells/mL	2.2 x 10 ⁷ cells/mL
Viability (post-freeze) ³	Growth	Growth
Sterility (21-day incubation) Harpo's HTYE broth ⁴ , 37°C and 26°C, aerobic Trypticase soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic Brain heart infusion, 37°C and 26°C, aerobic Sheep blood agar, 37°C, aerobic Sheep blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic	No growth	No growth

NR-42010 was produced by cultivation of the deposited material 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. The culture was propagated for 2 days at 27°C in an ambient atmosphere.

Date: 16 JAN 2014

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Signature:

Title:

Technical Manager, BEI Authentication or designee

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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BEI Resources E-mail: contact@beiresources.org

Tel: 800-359-7370 Fax: 703-365-2898

²PCR 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.

³Viable cells were seen after 4 days under cultivation conditions.

⁴Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.