

Certificate of Analysis for NR-42012

Trypanosoma brucei subsp. brucei, Strain Lister 427 29-13 (TetR T7RNAP) (procyclic form)

Catalog No. NR-42012

Product Description: *Trypanosoma brucei* (*T. brucei*) subsp. *brucei*, strain Lister 427 29-13 (TetR T7RNAP) was deposited to BEI Resources as a procyclic form cell line that expresses the T7 RNA polymerase (T7RNAP) and the tetracycline repressor (TetR) genes. Strain Lister 427 29-13 (TetR T7RNAP) was derived by sequential stable transfection of the procyclic form of wild-type *T. brucei* subsp. *brucei*, strain Lister 427. The parent strain Lister 427 is a virulent lab strain isolated in 1960 from a sheep in Uganda and transferred to the Lister Institute in London in 1961.

Lot¹: 61677338 Manufacturing Date: 02APR2013

TEST	SPECIFICATIONS	RESULTS
Genotyping Sequencing of 18S ribosomal RNA gene (~ 1590 base pairs) Sequencing of internal transcribed spacer (ITS) 1, 5.8S ribosomal RNA gene, ITS 2 (~ 930 base pairs)	Consistent with <i>T. brucei</i> Consistent with <i>T. brucei</i>	Consistent with <i>T. brucei</i> Consistent with <i>T. brucei</i>
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 amplicon	~ 2200 base pair amplicon ~ 1300 base pair amplicon
Presence of T7 RNA Polymerase and Tetracycline Repressor (Tetr/T7RNAP)	~ 1700 base pair amplicon	~ 1700 base pair amplicon
Viable Cell Count by Hemacytometry (pre-freeze)	> 10 ⁶ cells/mL	2.4 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

¹NR-42012 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 4 days at 27°C in an ambient atmosphere.

Date: 20 FEB 2014

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Signature:

Title:

Technical Manager, BEI Authentication or designee

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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." Exp Parasitol. 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.