b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-42011

Trypanosoma brucei subsp. *brucei*, Strain Lister 427 VSG 221 (TetR T7RNAP) (bloodstream form)

Catalog No. NR-42011

Product Description: *Trypanosoma brucei* (*T. brucei)* subsp. *brucei,* strain Lister 427 VSG 221 (TetR T7RNAP) was deposited to BEI Resources as a bloodstream form cell line that expresses the *T. brucei* variant surface glycoprotein (VSG) 221 (also referred to as MITat 1.2), T7 RNA polymerase (T7RNAP) and the tetracycline repressor (TetR) genes. The parental strain of Lister 427 VSG 221 (TetR T7RNAP), strain Lister 427 VSG 221, is a single-antigen isolate of strain Lister 427. 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.

Lot[1]: 61775530 **Manufacturing Date: 15MAY2013**

TEST	SPECIFICATIONS	RESULTS
Genotyping		
Sequencing of 18S ribosomal RNA gene (~ 1560 base pairs)	Consistent with *T. brucei*	Consistent with *T. brucei*
Sequencing of internal transcribed spacer (ITS) 1, 5.8S ribosomal RNA gene, ITS 2 (~ 930 base pairs)	Consistent with *T. brucei*	Consistent with *T. brucei*
Functional Activity by PCR Amplification[2]		
18S ribosomal RNA gene	~ 2200 base pair amplicon	~ 2200 base pair amplicon
ITS 1, 5.8S ribosomal RNA gene, ITS 2	~ 1300 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	1.8 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	No growth	No growth
Trypticase soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud broth, 37°C and 26°C, aerobic	No growth	No growth
Brain heart infusion, 37°C and 26°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, anaerobic	No growth	No growth
Thioglycollate broth, 37°C, anaerobic	No growth	No growth

[1]NR-42011 was produced by cultivation of the deposited material in modified HMI-9 medium. The culture was propagated in 95% air, 5% CO_2 for 2 days at 37°C.
[2]PCR 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.
[3]Viable cells were seen after 4 days under cultivation conditions.
[4]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Date: 20 FEB 2014 **Signature:** 

Title: Technical Manager, BEI Authentication or designee

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BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898