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

Catalog No. NR-42011

For research use only. Not for human use.

Contributor:
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Manufacturer:
BEI Resources

Product Description:
Protozoa Classification: *Trypanosomatidae*, *Trypanosoma*
Species: *Trypanosoma brucei* subsp. *brucei*
Strain: Lister 427 VSG 221 (TetR T7RNAP) (bloodstream form)
Original Source: *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.[1-3] The parental strain of Lister 427 VSG 221 (TetR T7RNAP), strain Lister 427 VSG 221 (BEI Resources NR-42009), is a single-antigen isolate of strain Lister 427.[1,2] 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.[3-5]
Comment: *T. brucei* subsp. *brucei,* strain Lister 427 VSG 221 (TetR T7RNAP) is a transgenic bloodstream form cell line that co-expresses the T7RNAP and TetR genes as well as VSG 221.[1-3].

T. brucei is a kinetoplastid protozoan parasite and is the causative agent of African trypanosomiasis, which is transmitted to both humans and livestock through the bite of the tsetse fly.[6,7] The flies inject the infective stage (metacyclic trypomastigotes) from their salivary glands into the blood and lymphatic fluid of the host, where they undergo differentiation and enter the central nervous system by evading the host immune system through the use of antigenic variation of their surface glycoprotein coat.[6,7]

T. brucei is divided into three morphologically-identical subspecies that display different pathogenicity: *T. brucei* subsp. *gambiense* and *T. brucei* subsp. *rhodesiense*, which cause African sleeping sickness in humans, and the non-human infective *T. brucei* subsp. *brucei*.[6,8] The production of vaccines against these diseases is difficult as a significant rise of resistance to trypanocidal drugs has been documented. Animal models such as mice are critical to understanding the mechanisms of disease and the development of new therapeutic drugs.[6]

T. brucei subsp. *brucei* transgenic bloodstream form cell lines are useful in the production of functional gene knock-outs through regulated expression of an experimental gene in a null-mutant background.[1]

Material Provided:
Each vial of NR-42011 contains approximately 0.5 mL of culture in 10% glycerol. Please see Appendix I for cryopreservation instructions.

Packaging/Storage:
NR-42011 was packaged aseptically in screw-capped plastic cryovials and is provided frozen on dry ice. The product should be stored at cryogenic temperature (-130°C or colder), preferably in the vapor phase of a liquid nitrogen freezer. If liquid nitrogen storage facilities are not available, frozen cryovials may be stored at -70°C or colder for approximately one week. Note: Do not under any circumstances store vials at temperatures warmer than -70°C. Storage under these conditions will result in the death of the culture.

To insure the highest level of viability, the culture should be initiated immediately upon receipt. Any warming of the product during shipping and transfer must be avoided, as this will adversely affect the viability of the product. For transfer between freezers and for shipping, the product may be placed on dry ice for brief periods, although use of a portable liquid nitrogen carrier is preferred. Please read the following recommendations prior to using this material.

Growth Conditions:
Modified HMI-9 medium (see Appendix II).
Incubation:
Temperature: 37°C
Atmosphere: 95% air, 5% CO_2
Propagation:
1. To establish a culture from the frozen state, place a vial in a 35°C to 37°C water bath. Thawing time is approximately 2 to 3 minutes. Do not agitate the vial. Do not leave the vial in the water bath after it is thawed.
2. Immediately after thawing, transfer the vial contents to a T-25 tissue culture flask containing 10 mL of HMI-9 medium. Incubate at 37°C with the cap screwed on tightly.
3. Observe the culture daily under an inverted microscope for the presence of bloodstream forms of the parasite. Subculture when the culture has reached peak density.

Maintenance:
1. Agitate a culture at or near peak density and aseptically transfer 0.5 to 1.0 mL into a new tissue culture flask with fresh growth medium.
2. Incubate the culture at 37°C with 5% CO_2 and examine daily under an inverted microscope.
3. Transfer every 3 to 7 days, as needed. Note that the transfer interval should be determined empirically as it is dependent on the quantity of the inoculum.

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Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Trypanosoma brucei* subsp. *brucei*, Strain Lister 427 VSG 221 (TetR T7RNAP) (bloodstream form), NR-42011."

Biosafety Level: 2

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

Disclaimers:

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APPENDIX I: CRYOPRESERVATION

1. Harvest trypanosomes from multiple culture flasks that have reached peak density and transfer to 15 mL plastic centrifuge tubes. Centrifuge at 800 x g for 10 min.
3. Remove all but 0.5 mL of the supernatant from each tube, resuspend the cell pellets, and pool them into a single tube.
4. Adjust the parasite concentration to 0.5 to 1.0 x 10^7 cells/mL using fresh growth medium or modified HMI-9 medium (Appendix II).
   Note: If the concentration of parasites is too low, centrifuge at 800 x g for 10 min and resuspend in a smaller volume of medium to yield the desired parasite concentration.
6. Mix equal volumes of parasite suspension and fresh medium containing 20% glycerol to yield a final concentration of 2.5 to 5 x 10^6 cells/mL in 10% glycerol. The freezing process should start 15 to 30 minutes following the addition of cryoprotective solution to the parasite suspension.
   Note: To prevent culture contamination, penicillin-streptomycin solution (ATCC® 30-2300) may be added to a final concentration of 50 to 100 U/mL penicillin and 50 to 100 µg/mL streptomycin.
7. Dispense 0.5 mL aliquots into 1 to 2 mL sterile plastic screw-capped vials for cryopreservation.
8. Place the vials in a controlled rate freezing unit. From room temperature, cool the vials at -1°C/min to -40°C. If the freezing unit can compensate for the heat of fusion, maintain rate at -1°C/min through this phase. At -40°C, plunge vials into liquid nitrogen. Alternatively, place the vials in a Nalgene 1°C freezing container. Place the container at -80°C for 1.5 to 2 hours and then plunge the vials into liquid nitrogen.
9. Store in either the vapor or liquid phase of a nitrogen refrigerator (-130°C or colder).

APPENDIX II: Modified HMI-9 Medium

1. Prepare the Hypoxanthine stock solution (see recipe below), filter-sterilize and freeze in 100 mL aliquots.
2. Prepare the 10X HMI-9 supplement (in the order listed in the recipe below), filter-sterilize and freeze in 100 mL aliquots.

Hypoxanthine Stock Solution	
NaOH	20.0 g
Distilled water	1,000 mL
Add Hypoxanthine	13.6 g

10X HMI-9 Supplement	
Bathocuproine Disulfonic Acid	280 mg
L-Cysteine	1820 mg
Pyruvic Acid	1100 mg
L-Cytosine	100 mg
2-Mercaptoethanol	140 µL
Distilled Water	1,000 mL

3. Aseptically prepare the Modified HMI-9 Medium by adding the components listed below to the Iscove's Modified Dulbecco's Medium (IMDM) in the following order:

IMDM (Life Technologies, #12440-046)	700.0 mL
Heat-Inactivated FBS	100.0 mL
Serum Plus™ (Sigma-Aldrich, #14008C)	100.0 mL
Hypoxanthine stock solution	10.0 mL
10X HMI-9 supplement	100.0 mL

Reference: http://tryps.rockefeller.edu/trypsru2_culture_media_preparation.html