Trypanosoma brucei subsp. *brucei*, Strain 427 1339 Cas9 TetR T7RNAP (bloodstream form)

Catalog No. NR-56793

For research use only. Not for use in humans.

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Manufacturer:
BEI Resources

Product Description:
Protozoa Classification: *Trypanosomatidae*, *Trypanosoma*
Species: *Trypanosoma brucei* subsp. *brucei*
Strain: 427 1339 Cas9 TetR T7RNAP (bloodstream form)
Original Source: *Trypanosoma brucei* (*T. brucei)* subsp. *brucei,* strain 427 1339 Cas9 TetR T7RNAP (bloodstream form) was deposited to BEI Resources as a bloodstream form, transgenic cell line expressing Cas9 nuclease, T7 RNA polymerase (T7RNAP) and tetracycline repressor (TetR) genes on plasmid pJ1339.[1,2] Strain 427 1339 Cas9 TetR T7RNAP (bloodstream form) was derived from strain Lister 427, a virulent lab strain that was isolated in 1960 from a sheep in Uganda and transferred to the Lister Institute in London in 1961.[3,4,5]
Comment: *T. brucei* subsp. *brucei,* strain 427 1339 Cas9 TetR T7RNAP (bloodstream form) is competent for both CRISPR/Cas9 genome editing and tetracycline-controlled inducible expression.[1] The complete genome of parental strain *T. brucei* subsp. *brucei*, strain Lister 427 has been sequenced (GenBank: UFQF00000000).

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.[7,8]

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 knockouts through regulated expression of an experimental gene in a null-mutant background.[9]

Material Provided:
Each vial of NR-56793 contains approximately 0.5 mL of cells in cryopreservative (10% glycerol). Please refer to Appendix I for cryopreservation instructions.

Packaging/Storage:
NR-56793 was packaged aseptically in screw-capped plastic cryovials and is provided frozen on dry ice. The product should be stored at -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 ensure 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 (Appendix II)
Incubation:
Temperature: 37°C
Atmosphere: Aerobic with 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 modified HMI-9 medium. Incubate at 37°C in an aerobic atmosphere with 5% CO_2.
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 mL to 1.0 mL into a new tissue culture flask with fresh growth medium.
2. Incubate the culture at 37°C in an ambient atmosphere 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.

Please refer to Appendix I for cryopreservation instructions.

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Citation:

Acknowledgment for publications should read "The following reagent was obtained through the BEI Resources, NIAID, NIH: *Trypanosoma brucei* subsp. *brucei*, Strain 427 1339 Cas9 TetR T7RNAP (bloodstream form), NR-56793."

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 (BMBL). 6th ed. Washington, DC: U.S. Government Printing Office, 2020.

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1. Sunter, J., Personal Communication.
2. Alves, A. A., et al. "Control of Assembly of Extra-Axonemal Structures: The Paraflagellar Rod of Trypanosomes." J. Cell. Sci. 133 (2020): jcs242271. PubMed: 32295845.
3. "Pedigrees of some *Trypanosoma brucei* Strains in Common Use and an Index of Lister 427 VSGs." Laboratory of Molecular Parasitology, The Rockefeller University, https://tryps.rockefeller.edu/trypsru2_pedigrees.html.
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APPENDIX I: CRYOPRESERVATION

1. To harvest the *Trypanosoma* culture, remove the media containing trypanosomes from infected culture flasks that have reached peak density and transfer to 15 mL plastic centrifuge tubes. Centrifuge at 800 × g for 10 min.
2. Remove all but 0.5 mL of the supernatant from each tube, resuspend the cell pellets, and pool them to a single tube.
3. Adjust the cell concentration to 0.5×10^7 to 1×10^7 cells/mL with fresh growth medium.
   Note: If the concentration of cells is too low, centrifuge at 800 × g for 10 minutes and resuspend in a smaller volume of fresh medium to yield the desired parasite concentration.
4. Mix equal volumes of parasite suspension and fresh medium containing 20% glycerol to yield a final concentration of 2.5×10^6 to 5×10^6 cells/mL in 10% glycerol. The freezing process should start 15 to 30 minutes following the addition of cryoprotective solution to the cell suspension.
   Note: To prevent culture contamination, penicillin-streptomycin solution (ATCC® 30-2300™) may be added to a final concentration of 50 IU/mL to 100 IU/mL penicillin and 50 µg/mL to 100 µg/mL streptomycin.
5. Dispense 0.5 mL aliquots into 1 to 2 mL sterile plastic screw-capped vials for cryopreservation.
6. 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 vials into liquid nitrogen.
7. Store in either the vapor or liquid phase of a nitrogen refrigerator (-130°C or colder).

APPENDIX II: MODIFIED HMI-9 MEDIUM[1]

1. Prepare the Hypoxanthine stock solution, filter-sterilize and freeze in 100 mL aliquots.

Hypoxanthine Stock Solution	
Distilled water	1,000 mL
NaOH	4 g
Add Hypoxanthine	13.6 g

2. Prepare the 10X HMI-9 supplement by adding components in the order listed below, filter-sterilize and freeze in 100 mL aliquots.

10X HMI-9 Supplement	
Bathocuproine Disulfonic Acid	280 mg
L-Cysteine	1820 mg
Pyruvic Acid	1100 mg
Uracil	100 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).

IMDM (Gibco™ 12440-046)	700 mL
Heat-Inactivated FBS	100 mL
Serum Plus™ (Sigma-Aldrich, Inc. 14008C)	100 mL
Hypoxanthine stock solution	10 mL
10X HMI-9 supplement	100 mL

Reference:

1. "Trypanosome Media Preparation Protocols." Laboratory of Molecular Parasitology, The Rockefeller University, https://tryps.rockefeller.edu/trypsru2_culture_media_preparation.html.

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