Trypanosoma brucei subsp. *brucei*, Strain TREU 927/4 (GUTat 10.1 Clone)

Catalog No. NR-41946

For research use only. Not for human use.

Contributor:

John Donelson, Professor Emeritus of Biochemistry, Carver College of Medicine, University of Iowa, Iowa City, Iowa, USA

Manufacturer:

BEI Resources

Product Description:

Protozoa Classification: *Trypanosomatidae*, *Trypanosoma*
Species: *Trypanosoma brucei* subsp. *brucei*
Strain: TREU 927/4 (GUTat 10.1 Clone)
Original Source: *Trypanosoma brucei* (*T. brucei)* subsp. *brucei*, strain TREU 927/4 (GUTat 10.1 clone) was derived from isolate GPAL/KE/70/EATRO 1534, which was obtained in 1970 from wild-caught *Glossina pallidipes* (tsetse flies) in Kiboko, Kenya.[1,2] The isolate was passaged 12 times in mice to generate the TREU 927/4 strain[2], which was subjected to 27 rapid syringe passages in mice to produce the GUTat 10.1 clone.[3]
Comment: *T. brucei* subsp. *brucei,* strain TREU 927/4 (GUTat 10.1) was deposited to BEI Resources as a reference strain and is being sequenced by the J. Craig Venter Institute (*Trypanosome brucei* Genome Project; PRJNA11756).[4]

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.[5,6] 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.[5,6]

T. brucei is divided into three morphologically-identical subspecies that demonstrate distinct pathogenicities: *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*.[5,7] 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.[5]

Strain TREU 927/4 (GUTat 10.1 clone) displays the full range of known phenotypes for *T. brucei*, including complete cyclical development within the tsetse fly, mating, and production of short stumpy forms during bloodstream infection in the mammalian host and displays the GUTat 10.1 antigen with relative stability.[2,3]

Material Provided:

Each vial of NR-41946 contains approximately 0.5 mL of culture in cryopreservative. Please see Appendix I for cryopreservation instructions.

Packaging/Storage:

NR-41946 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:

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 or Trypanosome Medium (ATCC® Medium 431)
Incubation:
Temperature: 27°C
Atmosphere: Ambient
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 SDM-79 medium. Incubate at 27°C with the cap screwed on tightly.
3. Observe the culture daily under an inverted microscope for the presence of procyclic 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 27°C with the cap screwed on tightly 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.

© 2013 American Type Culture Collection (ATCC). All rights reserved.

Citation:
Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Trypanosoma brucei* subsp. *brucei*, Isolate TREU 927/4 (GUTat 10.1 Clone), NR-41946."

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:
You are authorized to use this product for research use only. It is not intended for human use.

Use of this product is subject to the terms and conditions of the BEI Resources Material Transfer Agreement (MTA). The MTA is available on our Web site at www.beiresources.org.

While BEI Resources uses reasonable efforts to include accurate and up-to-date information on this product sheet, neither ATCC® nor the U.S. Government makes any warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. Neither ATCC® nor the U.S. Government warrants that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, use and disposal. ATCC® and the U.S. Government are not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to ensure authenticity and reliability of materials on deposit, the U.S. Government, ATCC®, their suppliers and contributors to BEI Resources are not liable for damages arising from the misidentification or misrepresentation of products.

Use Restrictions:
This material is distributed for internal research, non-commercial purposes only. This material, its product or its derivatives may not be distributed to third parties. Except as performed under a U.S. Government contract, individuals contemplating commercial use of the material, its products or its derivatives must contact the contributor to determine if a license is required. U.S. Government contractors may need a license before first commercial sale.

References:
1. Goedbloed, E., et al. "Serological Studies of Trypanosomiasis in East Africa. II. Comparisons of Antigenic Types of *Trypanosoma brucei* Subgroup Organisms Isolated from Wild Tsetse Flies." Ann. Trop. Med. Parasitol. 67 (1973): 31-43. PubMed: 4723213.
2. Gibson, W. "The Origins of the Trypanosome Genome Strains *Trypanosoma brucei brucei* TREU 927, *T. b. gambiense* DAL 972, *T. vivax* Y486 and *T. congolense* IL3000." Parasit. Vectors 5 (2012): 71. PubMed: 22483376.
3. van Deursen, F.J., et al. "Characterisation of the Growth and Differentiation In Vivo and In Vitro of Bloodstream-Form *Trypanosoma brucei* Strain TREU 927." Mol. Biochem. Parasitol. 112 (2001): 163-171. PubMed: 11223123.
4. Berriman, M. et al. "The Genome of the African Trypanosome *Trypanosoma brucei*." Science 309 (2005): 416-422. PubMed: 16020726.
5. Antoine-Moussiaux, N., et al. "Contributions of Experimental Mouse Models to the Understanding of African Trypanosomiasis." Trends Parasitol. 24 (2008): 411-418. PubMed: 18684669.
6. Peacock, L., et al. "Identification of the Meiotic Life Cycle Stage of *Trypanosoma brucei* in the Tsetse Fly." Proc. Natl. Acad. Sci. USA 108 (2011): 3671-3676. PubMed: 21321215.
7. Turner, C. M., et al. "Replication, Differentiation, Growth and the Virulence of *Trypanosoma brucei* Infections." Parasitology. 111 (1995): 289-300. PubMed: 7567097.

ATCC® is a trademark of the American Type Culture Collection.

© 2013 American Type Culture Collection (ATCC). All rights reserved.

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.
   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).

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

© 2013 American Type Culture Collection (ATCC). All rights reserved.