Product Information Sheet for NR-44388

Trypanosoma brucei subsp. *brucei*, Strain STIB 247 (*in vitro*)

Catalog No. NR-44388

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: STIB 247
Original Source: *Trypanosoma brucei* (*T. brucei)* subsp. *brucei*, strain STIB 247 (*in vitro*) was harvested from the blood of infected BALB/c mice and adapted to cell culture by BEI Resources. The parent strain STIB 247 (BEI Resources NR-36197) was isolated in 1971 from a hartebeest in Serengeti National Park, Serengeti, Tanzania, and prior to freezing, was passaged in rats for an unknown number of days.[1,2]

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.[3,4] 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.[3,4]

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*.[3,5] 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 the disease and also aid in the development of new therapeutic drugs.[3]

T. brucei subsp. *brucei*, strain STIB 247 is a parental strain that has been used in a genetic cross with *T. brucei* subsp. *brucei*, strain STIB 386 (BEI Resources NR-36198) to generate cloned lines expressing resistance to anti-parasitic drugs and to investigate genetic exchange in a mouse model.[6,7]

Material Provided:

Each vial of NR-44388 contains approximately 0.5 mL of culture in cryopreservative. Please see Appendix I for cryopreservation instructions.

Packaging/Storage:

NR-44388 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; see Appendix II)
Incubation:
Temperature: 27°C
Atmosphere: Aerobic
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 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 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.

Please see Appendix I below for cryopreservation instructions.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through the BEI Resources, NIAID, NIH: *Trypanosoma brucei* subsp. *brucei*, Strain STIB 247 (*in vitro*), NR-44388."

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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References:

1. Geigy, R. and M. Kauffmann. "Sleeping Sickness Survey in the Serengeti Area (Tanzania) 1971. I. Examination of Large Mammals for Trypanosomes." Acta Trop. 30 (1973): 12-23. PubMed: 4144952.
2. http://tryps.rockefeller.edu
3. Antoine-Moussiaux, N., et al. "Contributions of Experimental Mouse Models to the Understanding of African Trypanosomiasis." Trends Parasitol. 24 (2008): 411-418. PubMed 18684669.
4. 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.
5. Turner, C. M., et al. "Replication, Differentiation, Growth and the Virulence of *Trypanosoma brucei* Infections." Parasitology 111 (1995): 289-300. PubMed: 7567097.
6. Scott, A. G., et al. "Characterisation of Cloned Lines of *Trypanosoma brucei* Expressing Stable Resistance to MelCy and Suramin." Acta Trop. 60 (1996): 251-262. PubMed: 8659324.
7. Jenni, L., et al. "Hybrid Formation between African Trypanosomes during Cyclical Transmission." Nature 322 (1986): 173-175. PubMed: 3724860.

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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 x 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 2 x 10^7 to 4 x 10^7 cells/mL with fresh Modified M199 medium.
   Note: If the concentration of cells is too low, centrifuge at 800 x 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 x 10^7 to 5 x 10^7 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 to 100 IU/mL penicillin and 50 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: ATCC® medium: 431 (*Trypanosome* medium)

Solid Phase:
Beef extract.....3.0 g
Peptone.....5.0 g
NaCl.....8.0 g
Agar.....15.0 g
Distilled water.....1.0 L
Liquid Phase (Locke's solution):
NaCl.....8.0 g
KCl.....0.2 g
$CaCl_2$ .....0.2 g
KH_2PO_4 .....0.3 g
Glucose.....2.5 g
Distilled water.....1.0 L

Adjust pH of both phases to 7.2 to 7.4. Autoclave both phases at 121°C for 15 minutes. Cool the solid phase mixture to about 45°C and aseptically add 30% sterile, defibrinated, rabbit blood. Aseptically dispense in sterile 16 X 125 mm screw-capped test tubes in 5 mL amounts and cool on a slant. After cooling the slants, aseptically dispense 3.0 mL of Locke's solution (Liquid phase) over each slant.

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