Product Information Sheet for NR-46440

Trypanosoma brucei subsp. *brucei*, Strain TREU 667

Catalog No. NR-46440

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: TREU 667
Original Source: *Trypanosoma brucei* (*T. brucei*) subsp. *brucei*, strain TREU 667 (Trypanosomiasis Research Edinburgh University) was originally isolated in Kenya.[1]
Comments: The TREU 667 strain is a pleomorphic strain of low virulence to mice. It had been passaged once in rats after its receipt in Edinburgh, where it was established as a stabilate and kept in low temperature storage.[1] *T. brucei* subsp. *brucei*, strain TREU 667 was obtained by Professor C. J. Bacchi from F. W. Jennings at University of Glasgow, United Kingdom.[2] In mice, the TREU 667 strain produces a semi-acute infection lasting 60 to 70 days; this model of progressive pathology is more similar to natural infections in humans and domestic animals than acute models, which are lethal within a few days.[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.[4,5] 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.[4,5]

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*.[4,6] 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.[4]

Material Provided:

Each vial of NR-46440 contains approximately 0.5 mL of trypanosome infected blood with 20% glycerol, 6.25% Yaeger's anticoagulant and Trypanosome dilution buffer. Please see Appendix I for cryopreservation instructions and component details.

Packaging/Storage:

NR-46440 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:

In vivo, BALB/c mouse
Inoculation:

1. Thaw a frozen ampule of NR-46440 in a 35°C to 37°C water bath for approximately 2 to 3 minutes.
2. Remove the contents of the ampule using a 1 mL syringe equipped with a 27 gauge 1/2 inch needle.
3. Inject the entire contents of the vial intraperitoneally into a mouse.

Monitoring parasitemia:

1. Bleed the mouse at 2- to 3-day intervals to monitor parasitemia by microscopic examination using a haemocytometer and 0.85% ammonium chloride as diluent. Parasitemia may also be assessed by microscopic examination of blood films stained with a 5% Giemsa solution.
2. Passage the strain when the infection is at or near the first peak of parasitemia (≥ $5x10^5$ parasites/mL or ≥ 5 parasites/high power field for Giemsa-stained blood films observed under 100X). This will normally occur after 5 to 7 days of inoculation. Note that the rate of *T. brucei* subsp. *brucei* infection may vary with the parasite strain and concentration of inoculum.

Passaging:

1. Anesthetize the infected mouse by CO_2/O_2 inhalation. Collect the blood by orbital bleeding or from the tail vein using an anticoagulant such as Yaeger's anticoagulant solution (Appendix I) or EDTA.
2. Perform a parasite count and inject $5x10^4$ to $1x10^5$ parasites into each of the uninfected mice (approximately 10 mice).
3. Monitor parasitemia as described above and passage as needed.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Trypanosoma brucei* subsp. *brucei*, Strain TREU 667, NR-46440."

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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3. Amole, B. O., A. B. Clarkson, Jr. and H. Lustig Shear. "Pathogenesis of Anemia in *Trypanosoma brucei*-Infected Mice." Infect. Immun. 36 (1982): 1060-1068. PubMed: 7201455.
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5. 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.
6. Turner, C. M., N. Aslam and C. Dye. "Replication, Differentiation, Growth and the Virulence of *Trypanosoma brucei* Infections." Parasitology 111 (1995): 289-300. PubMed: 7567097.

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APPENDIX I: CRYOPRESERVATION

1. Prepare a 40% (v/v) sterile glycerol solution in Trypanosome dilution buffer (see below).
2. Dispense 0.5 mL of anticoagulant solution (see below) into a 15 mL test tube. Add to the anticoagulant tube blood collected by orbital bleeding from mice that had reached or are near peak parasitemia. Invert the tube several times to mix the blood with the anticoagulant.
3. In a separate test tube, add the heparinized blood dropwise to the 40% glycerol solution. Note that blood should be mixed with glycerol solution in a 1:1 ratio to obtain a final concentration of cryoprotectant of 20% (v/v). Mix slowly by inversion and place the tube on ice. The freezing process should start 15 to 30 minutes following the addition of the heparinized blood to the cryoprotectant solution.
4. Dispense 0.5 mL aliquots of blood suspension into 1 to 2 mL sterile plastic screw-capped cryovials. 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.
5. To thaw a frozen ampule, place in a 35°C to 37°C water bath, until thawed (2 to 3 min). Immerse the ampule just sufficient to cover the frozen material. Do not agitate the ampule.
6. Immediately after thawing, remove the contents of the ampule aseptically with a syringe and inoculate an immunosuppressed mouse. Follow the protocol for in vivo propagation above.

Trypanosome dilution buffer
20 mM Na_2HPO_4
2 mM NaH_2PO_4
80 mM NaCl
5 mM KCl
1 mM $MgSO_4$
20 mM Glucose

Adjust the pH of the solution to 7.7 and filter-sterilize.

Yaeger's anticoagulant solution

Sodium citrate	1.33 g
Citric acid	0.47 g
Dextrose	3.00 g
Sodium heparin	0.20 g
Distilled H_2O to	100.0 mL