Strain Designation: TREU 667
Depositor: C.J. Bacchi
Isolation: Unknown

Growth Conditions
Culture System: *in vivo*, Balb/c mouse

Storage and Culture Initiation
Frozen ampules packed in dry ice should either be thawed immediately or stored in liquid nitrogen. If liquid nitrogen storage facilities are not available, frozen ampoules may be stored at or below -70°C for approximately one week. **Do not under any circumstance store frozen ampules at refrigerator freezer temperatures (generally -20°C).** Storage of frozen material at this temperature will result in the death of the culture.

1. To thaw a frozen ampule, place it in a 35°C water bath such that the lip of the ampule remains above the water line. Thawing time is approximately 2 to 3 minutes. Do not agitate the ampule. Do not leave ampule in water bath after it is thawed.

2. Immediately after thawing, aseptically remove the contents of the ampule with a syringe and inoculate a Balb/c mouse. Follow the protocol for maintenance of the culture below. The course of infection may depend on the parasite strain and recovery from the frozen state.

Culture Maintenance
Yaeger’s anticoagulant
Sodium citrate, 1.33 g
Citric acid, 0.47 g
Dextrose, 3.00 g
Sodium heparin, 0.20 g
Glass distilled H₂O, to 100.00 mL

1. Inoculate entire infected blood suspension intraperitoneally into a Balb/c mouse using a 1.0 mL syringe equipped with a 27 gauge 1/2 inch needle.

2. Bleed the mouse at 2-3 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 5% Giemsa solution.

3. Passage the strain when the infection is at a parasitemia of ≥ 5 x 10⁵ parasites/mL or ≥ 5 parasites/HPF for Giemsa-stained blood films observed under 100X. This will normally occur after 5-7 days of inoculation. Note that the rate of *T. brucei brucei* infection may vary with the parasite strain.

4. To passage the strain, anesthetize the first infected mouse by CO₂/O₂ inhalation. Collect the blood by orbital bleeding or from the tail vein using an anticoagulant such as Yaeger’s anticoagulant solution or EDTA.

5. Perform a parasite count and inject 5 x 10⁴ to 1 x 10⁵ parasites into a determined number of Balb/c mice (~10).

6. Monitor parasitemia as described above and passage as needed.

**NOTE:** Cardiac puncture may be used as an alternative method of blood collection.

Cryopreservation
Reagents
Trypanosome Dilution Buffer
20 mM Na₂HPO₄
2 mM NaH₂PO₄
80 mM NaCl
5 mM KCl
1 mM MgSO₄
20 mM glucose

Adjust the pH of the solution to 7.7 and filter sterilize.
Harvest and Preservation

1. Prepare a 40% (v/v) sterile glycerol solution in Trypanosome Dilution Buffer (TDB).
2. Dispense 0.5 mL of anticoagulant solution into a 15 mL test tube. Add to the anticoagulant 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 1.0 - 2.0 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 throughout 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 water bath, until thawed (2-3 min). Immerse the ampule just sufficient to cover the frozen material. Do not agitate the ampule.
6. Immediately after thawing, aseptically remove the contents of the ampule with a syringe and inoculate a Balb/c mouse. Follow the protocol for maintenance of the culture above.

References

References and other information relating to this product are available online at www.atcc.org.

Biosafety Level: 2

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the Biosafety in Microbiological and Biomedical Laboratories from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

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