Trypanosoma brucei rhodesiense Stephens and Fantham

PRA-408™

Description

Strain designation: KETRI 2538
Deposited As: *Trypanosoma brucei rhodesiense*

Storage Conditions

Product format: Frozen
Storage conditions: -80°C or colder for 1 week, vapor phase of liquid nitrogen for long-term storage

Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

BSL 2

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local or national agencies.

ATCC highly recommends that appropriate personal protective equipment is always used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitrogen.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Growth Conditions

Host:

in vivo cultivation, Balb/c mouse

Handling Procedures

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 equipped with a 27 gauge 1/2 inch needle.
3. Inject 100 µL of the contents or 0.1 mL of the contents of the vial intra-peritoneally (i.p.) into a Balb/c mouse. Inject the remaining contents into a second 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 H_2O to 100.00 mL

1. Bleed the mice at daily 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.
2. Passage the strain when the infection is at or near the first peak of parasitemia (≥ 1 x 10^8 parasites/mL (≥ 30 parasites/HPF for Giemsa-stained blood films observed under 100X). This will normally occur within 4 days of inoculation. Note that the rate of *T. brucei rhodesiense* infection may vary with the parasite strain and concentration of inoculum.
3. To passage the strain, 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 or EDTA.
4. Perform a parasite count and inject 1 x 10^6 to 1 x 10^7 parasites into a determined number of Balb/c mice (~10).
5. Monitor parasitemia as described above and passage as needed.

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

Reagents for cryopreservation: 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.

Cryopreservation:

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 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 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.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Trypanosoma brucei rhodesiense* Stephens and Fantham (ATCC PRA-408)

References

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

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