Trypanosoma brucei brucei

PRA-421™

Description

Strain designation: TREU 667 *in vitro* procyclic form
Deposited As: *Trypanosoma brucei brucei*

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

Medium:
ATCC Medium 2831: SDM-79 Medium
ATCC Medium 431: Trypanosome medium
Instructions for complete medium: Media: ATCC® Medium 2831: SDM-79 Medium

Alternate Media: ATCC® Medium 431: Trypanosome medium
Temperature: 27°C
Atmosphere: Ambient
Culture system: Axenic

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 ampules may be stored at or below -70ºC 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 in a 35ºC water bath, until thawed (2-3 min). Immerse the ampule just sufficiently to cover the frozen material. Do not agitate the ampule.
2. Immediately after thawing, aseptically transfer contents to a T-25 tissue culture flask containing 10.0 mL of growth medium. Incubate at 27°C with the cap screwed on tightly.

Culture maintenance:

1. Agitate a culture at or near peak density and aseptically transfer 0.5-1 mL to a new tissue culture flask with fresh growth medium.
2. Incubate at 27°C with the cap screwed on tightly.
3. Transfer the culture every 3-7 days as described in steps 1-2. The transfer interval will depend on the quantity of the inoculum and the quality of the medium. This should be empirically determined by examining the culture on a daily basis until the growth cycle has stabilized.

Cryopreservation:

1. Harvest cells from a culture which is at or near peak density by centrifugation at ~800 x g for 5 min.
2. Adjust concentration of cells to 0.5–1.0 x 10^7/mL in fresh growth medium. If the concentration is too low, centrifuge at ~800 x g for 5 minutes and resuspend the cell pellet with a volume of supernatant to yield the desired concentration.
3. While cells are centrifuging, prepare a 20% (v/v) solution of sterile dimethyl sulfoxide (DMSO) in fresh growth medium.
4. Mix the cell preparation and the DMSO solution in equal portions. The final concentration will be 2.5-5 x 10^6 cells/mL in 10% DMSO. The time from the mixing of the cell preparation and the DMSO stock solution before the freezing process is begun should be no less than 15 min and no more than 30 min.
5. Dispense in 0.5 mL aliquots into 1.0 - 2.0 mL sterile plastic screw-capped cryovials.
6. Place the ampules in a Nalgene 1°C freezing apparatus. Place the apparatus at -80°C for 1.5 to 2 hours and then plunge ampules into liquid nitrogen. (The

cooling rate in this apparatus is approximately -1°C/min.) If freezing unit can compensate for the heat of fusion, maintain rate at -1°C/min through heat of fusion. At -40°C, plunge ampules into liquid nitrogen.

7. Store in either the vapor or liquid phase of a nitrogen refrigerator.
8. 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.
9. Remove the vial from the water bath immediately after thawing. Aseptically transfer the contents of the ampule into 10 mL of fresh growth medium.
10. Incubate the flask at 27°C with the cap screwed on tightly.
11. Maintain as described above.

NOTE: DMSO may be substituted with glycerol as an alternative cryoprotectant.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Trypanosoma brucei brucei* (ATCC PRA-421)

References

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

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Contact Information

ATCC
10801 University Boulevard
Manassas, VA 20110-2209
USA
US telephone: 800-638-6597
Worldwide telephone: +1-703-365-2700
Email: tech@atcc.org or contact your local distributor