



Trypanosoma brucei Plimmer and Bradford

PRA-381™

Description

Strain designation: Lister 427 29-13 (TetR T7RNAP) transgenic procyclic form

Deposited As: *Trypanosoma brucei* Lister 427 29-13 transgenic procyclic form

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 submerged 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 submerged 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 should be completed by the addition of 10% HIFBS and 7.5 µg/mL hemin just prior to use.

Alternate Media: ATCC Medium 431: Trypanosome medium

Temperature: 27°C

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 -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 in a 35°C 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 $\sim 800 \times g$ for 5 min.
2. Adjust concentration of cells to $0.5\text{--}1.0 \times 10^7/\text{mL}$ in fresh growth medium. If the concentration is too low, centrifuge at $\sim 800 \times g$ 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 glycerol in fresh growth medium.
4. Mix the cell preparation and the glycerol solution in equal portions. The final concentration will be $2.5\text{--}5 \times 10^6$ cells/mL in 10% glycerol. The time from the mixing of the cell preparation and glycerol 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 $-1^{\circ}\text{C}/\text{min}$.) If freezing unit can

compensate for the heat of fusion, maintain rate at $-1^{\circ}\text{C}/\text{min}$ 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.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Trypanosoma brucei* Plimmer and Bradford (ATCC PRA-381)

References

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

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