Appendix I, II for *Trypanosoma cruzi*

APPENDIX I: CRYOPRESERVATION

1. To harvest the *Trypanosoma* culture, remove the media containing trypanosomes from infected culture flasks that have reached peak density and transfer to 15 mL plastic centrifuge tubes. Centrifuge at 800 × g for 10 minutes.
2. Remove all but 0.5 mL of the supernatant from each tube, resuspend the cell pellets and pool them into a single tube.
3. Adjust the parasite concentration to 2×10^7 to 4×10^7 cells/mL using fresh growth medium.
   Note: If the concentration of parasites is too low, centrifuge at 1300 × g for 10 minutes and resuspend in a smaller volume of fresh medium to yield the desired parasite concentration.
4. Mix equal volumes of parasite suspension and fresh medium containing 10% dimethylsulfoxide (DMSO) to yield a final concentration of 1×10^7 to 2×10^7 cells/mL in 5% DMSO. The freezing process should start 15 to 30 minutes following the addition of cryoprotective solution to the parasite suspension.
   Note: To prevent culture contamination, penicillin-streptomycin solution (ATCC® 30-2300™) may be added to a final concentration of 50 IU/mL to 100 IU/mL penicillin and 50 IU/mL to 100 IU/mL to 100 IU/mL streptomycin.
5. Dispense 0.5 mL aliquots into 1 mL to 2 mL sterile plastic screw-capped vials for cryopreservation.
6. Place the vials in a controlled rate freezing unit. From room temperature cool the vials at -1°C/minute to -40°C. If the freezing unit can compensate for the heat of fusion, maintain rate at -1°C/minute 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.
7. Store in either the vapor or liquid phase of a nitrogen refrigerator (-130°C or colder).

APPENDIX II: ATCC® MEDIUM 1029

Liver Infusion Broth (BD 226920)	9 g
Tryptose (BD 211713)	5 g
NaCl	1 g
Na_2HPO_4	8 g
KCl	0.4 g
Glucose	1 g
Fetal bovine serum (heat-inactivated)	100 mL
Hemin	10 mg
Distilled water to	1 L

Adjust pH to 7.2 and filter-sterilize. Dispense aseptically in 5 mL aliquots into 16 × 125 screw-capped test tubes.

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www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898