

APPENDIX I: CRYOPRESERVATION

1. To harvest *Trypanosoma cruzi*, remove the media containing trypomastigotes from a series of infected cultures in T75 flasks and transfer to 15 mL plastic centrifuge tubes. Centrifuge at $1300 \times g$ 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 $1300 \times g$ 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 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 $-1^\circ\text{C}/\text{minute}$ to -40°C . If the freezing unit can compensate for the heat of fusion, maintain rate at $-1^\circ\text{C}/\text{minute}$ 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).