

APPENDIX I: CRYOPRESERVATION

1. To harvest the *Toxoplasma* culture, detach any remaining tissue culture cells (infected and uninfected) by scraping the surface of the flask with a cell scraper.
2. Transfer the cell suspension (including parasites) to 15 mL plastic centrifuge tubes. Centrifuge at 1300 × g for 10 minutes.
3. Remove all but 0.5 mL of the supernatant from each tube, resuspend the cell pellets and pool them to a single tube.
4. Pass the resulting cell suspension through a syringe equipped with a 27-gauge ½-inch needle to break up any remaining cells.
5. Adjust the parasite concentration to 2×10^7 to 4×10^7 cells/ mL with fresh medium [DMEM containing 10% (v/v) HIFBS or Dulbecco's PBS (ATCC® 30-2200™) can be used].
Note: If the concentration of parasites is too low, centrifuge at 1300 x g for 10 minutes and resuspend in a smaller volume of fresh medium to yield the desired parasite concentration.
6. Mix equal volumes of parasite suspension and fresh medium or PBS containing 15% DMSO and 50% HIFBS to yield a final concentration of 1×10^7 to 2×10^7 cells/mL in 7.5% DMSO, 25% HIFBS. 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 µg/mL to 100 µg/mL streptomycin.
7. Dispense 0.5 mL aliquots into 1 mL to 2 mL sterile plastic screw-capped vials for cryopreservation.
8. Place the vials in a controlled rate freezing unit. From room temperature, cool the vials at -1°C per minute to -40°C. If the freezing unit can compensate for the heat of fusion, maintain rate at -1°C per 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.
9. Store in either the vapor or liquid phase of a nitrogen refrigerator (-130°C or colder).