APPENDIX: CRYOPRESERVATION OF *TRICHOMONAS* CULTURES

1. Harvest cells from several *Trichomonas* cultures that are in peak density of growth and place on ice for 10 minutes.
2. Gently invert tubes several times and centrifuge at 800 × g for 5 minutes.
3. While cells are centrifuging, prepare the Cryoprotective Solution [10% (v/v) solution of sterile DMSO in fresh medium]:
    a. Add 1 mL of DMSO to a 20 × 150 mm screw-capped test tube and place on ice until solidified (approximately 5 minutes).
    b. Add 9 mL of ice cold incomplete modified TYM medium and invert until the DMSO is liquefied.
    c. Allow the solution to warm to room temperature.
4. Resuspend the cell pellets and pool to a final volume of approximately 10 mL with fresh complete Modified TYM Basal medium supplemented with 10% HIHS and 25 mM iron.
5. Determine the cell density using a hemocytometer and adjust the concentration to between 2×10^6 to 2×10^7 cells/mL with fresh complete Modified TYM Basal medium supplemented with 10% HIHS and 25 mM iron.
   Note: If the concentration of cells is too low, centrifuge at 800 × g for 10 minutes and resuspend in a smaller volume of fresh medium to yield the desired parasite concentration.
6. Mix equal volumes of cell suspension and the Cryoprotective Solution prepared above to yield a final concentration of 1×10^6 to 1×10^7 cells/mL in 5% DMSO. The freezing process should start 15 to 30 minutes following the addition of cryoprotective solution to the cell 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 cryovials.
8. Place the vials in a controlled rate freezing unit. From room temperature, cool at -10°C per minute until the liquid begins to freeze; from this point until -40°C is reached, cool at -1°C per minute. At -40°C plunge the vials into liquid nitrogen. The cooling cycle should be initiated 15 to 30 minutes after the addition of DMSO to the cell preparation.
9. Store in either the vapor or liquid phase of a nitrogen refrigerator (-130°C or colder).

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