Freezing Down Cultures for Frozen Stocks

Reagents:

- Deep-Freezing Solution (100mL)

    1) To 50mL ddH_20 add…
    2) 28mL glycerol (28% v/v)
    3) 3g sorbitol (3% w/v)
    4) 0.65g NaCl (0.65% w/v)
    5) Add ddH_20 to bring volume to 100mL.
    6) Sterilize by 0.22μm filtration, store at 4.0°C.

Procedure:

1. Select a stock culture of approximately 5% parasitemia containing a high proportion of ring stage parasites. (Trophozoites and schizonts are destroyed when frozen). A synchronized culture may be used.
2. Resuspend 5 mL of culture and spin at 800xg for 5 min. (Don't forget to set the brake to low).
3. Remove the supernatent from the cell pellet, which should measure 300-500μL in volume. This volume is enough for two cryovials.
4. Add an equal volume of deep-freezing solution to the cell pellet drop by drop at RT to allow the glycerol to penetrate the cells.
5. Place a final volume and not more than 500μL into each cryovial (200μL is sufficient).
6. Freeze and store immediately in liquid nitrogen.