

## Freezing Down Cultures for Frozen Stocks

### Reagents:

- Deep-Freezing Solution (100mL)
  - 1) To 50mL ddH<sub>2</sub>O add...
  - 2) 28mL glycerol (28% v/v)
  - 3) 3g sorbitol (3% w/v)
  - 4) 0.65g NaCl (0.65% w/v)
  - 5) Add ddH<sub>2</sub>O 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 supernatant 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.