

# Synchronization Method

(Updated 2008)

- This method is designed to eliminate the trophozoite and schizont stages hence selecting for ring-stage parasites.
  - The most important thing for synchronization is to be sure that you have enough ring-stage parasites.
  - For tighter synchronization, this method should be done several times until the ring-stage predominates in the cultures.
- 1) Make a 5 % Sorbitol (sterile-filtered) solution in PBS. Pre-warm for 15 minutes in 37°C water bath. (It can be stored long-term at 4 °C.)
  - 2) Transfer 50 mL of ring-stage culture to a 50 mL conical tube and spin the cells down in a tabletop centrifuge at 1500 RPM for 5 minutes, low brake.
  - 3) Aspirate off the media.
  - 4) Take 8.0 ul of culture and do a smear on a microscope slide for analysis of parasitaemia and to check the general synchrony of the culture.
  - 5) Resuspend cells in a total of 50 mL of 5% Sorbitol per 3 flasks (general rule of thumb) and transfer to one 50 mL conical tube. Mix well and incubate the cells for 10 minutes at room temperature.
  - 6) Spin the cells out of the Sorbitol solution in a tabletop centrifuge at 1500 RPM for 5 minutes, low brake.
  - 7) Aspirate off the supernatant.
  - 8) Wash the cells by resuspending in 50 mL media and spinning down in a tabletop centrifuge at 1500 RM for 5 minutes, low brake.
  - 9) Aspirate off the supernatant.
  - 10) Resuspend the cells in media such that when you add back to the flasks the total volume is 50 mL of culture.
  - 11) Place back into the incubator.