Parasite Extraction

Reagents:

- Parasite cultures (~250mL of 8-12% parasitemia culture)
- 15mL and 50mL sterile tubes
- Phosphate-Buffered Saline (PBS); [Gibco]
- 1.5% Saponin stock solution; [Acros]

Protocol:

1) Re-suspend parasite culture and transfer to 50 mL sterile tubes.
2) Centrifuge 800xg for 5' @ 4°C.
3) Remove the supernatent and bring volume to 40mL using PBS. Re-suspend the cells.
4) Centrifuge 800xg for 5' @ 4°C.
5) Remove the supernatent and add 5 pellet volumes of 0.15% Saponin, mix by pipetting until the color changes to a dark red indicating red blood cell lysis.
6) Leave on ice 10-15' after homogenation.
7) Centrifuge 3200xg 10' @ 4°C.
8) Remove the supernatent and add 2 volumes of 0.15% saponin, then bring volume to 40mL total using PBS. Mix slowly by pipetting and leave on ice 5'.
9) Centrifuge 3200xg 10' @ 4°C.
10) Combine pellets into one 15mL sterile tube and bring volume to 15mL using PBS.
11) Centrifuge 3200xg 10' @ 4°C.
12) Remove the supernatent and aliquot pellet into microcentrifuge tubes.
13) Fill microcentrifuge tubes with PBS and centrifuge @ 3200xg 5' @ 4°C.
14) Remove supernatent and freeze parasite pellets on liquid nitrogen while in microcentrifuge tubes. The final pellet of pure parasites should be black and 250μL-1mL in size depending on amount and parasitemia of starting culture.
15) Store parasite pellets @ -80°C.