

## 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 supernatant and bring volume to 40mL using PBS. Re-suspend the cells.
- 4) Centrifuge 800xg for 5' @ 4°C.
- 5) Remove the supernatant 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 supernatant 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 supernatant and aliquot pellet into microcentrifuge tubes.
- 13) Fill microcentrifuge tubes with PBS and centrifuge @ 3200xg 5' @ 4°C.
- 14) Remove supernatant 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.