Extracellular Immunophenotyping for Flow Cytometry (PB or BM)

1. Pipette 0.5 to 1 x 10e6 cells into each tube needed for flow panel.
2. Add 100 µl of staining media (1X PBS -w/o ca and mg, with 3% FCS and 2mM EDTA) to each tube.
3. Vortex sample
4. Add the appropriate amount of each antibody to each tube (see "Titering Directly Conjugated Antibodies to Extracellular Antigens" pdf). Vortex.
5. Incubate samples in the dark for 15 minutes.
6. Pipette 2mL of ammonium chloride lysing Solution, keep samples at 4°c for 10 minutes

   ammonium chloride lysing solution preparation:

   1 liter of dH20

   8.26 g NH_4Cl (sigma# A5666)

   1.00 g $KHCO_3$ (sigma# P4913)

   0.037 g EDTA (sigma# E1644)

   Add all dry reagents to dH2O, mix until dissolved

   Adjust pH to 7.4

   Filter sterilize, store at 4°c for up to 4 months

7. Add 2 mL staining media, vortex and centrifuge for 5 minutes at 1300 rpm.
8. Remove supernatant, vortex remaining cell button (can repeat washing step #7 to clean up residual red cell debris in the sample).
9. Tubes stained with directly conjugated abs can be either fixed with 1% Paraformaldehyde 250 µl or add 250 µl of staining media if running fresh. Keep completed tubes refrigerated in the dark until ready to run flow.

   If using biotinylated ab:

   -Add 100 µl of staining media at this point instead. Add appropriate volume of conjugated streptavidin and incubated for 20 minutes.

   -Add 500 µl of staining media, mix well, then centrifuge.

   -Remove supernatant, vortex remaining cell button.

   -Add 250 µl of either 1% paraformaldehyde or staining media as in step #9.
10. Run on flow cytometer within 4 hours if fresh samples or store in dark/cold and run up to 4 days later if fixed with paraformaldehyde.