Flow Cytometry Staining Protocol
Cell Sorting

Supplies

- ACK
- 5 mL FACS tubes (Falcon)
- Block (2.4G2)
- FACS Staining Buffer (1XPBS w/ 3% calf serum and 0.05% sodium azide)
- Sorting Buffer (1xPBS w/ 0.1% BSA or 0.5% FCS)
- Collection Buffer (depends on application, RPMI or PBS/serum)
- 70uM filter: either BD #352350 sterile cell strainer cap (fits 50ml tube) or BD Falcon #352235
- 12x75 5ml tube with 70uM filter cap, nonsterile

Protocol

1. Remove spleens, LN, etc. into media on ice. Disrupt into single cell suspension using your favorite technique and pass through 70uM filter.
2. Spin 10 min. @ 1500 RPM, 8°C.
3. Perform red blood cell lysis, per lab protocol (either ACT, ACK or LSM). Resuspend in FACS staining buffer. (Use this buffer also for all washes until directed to use Sorting Buffer.) Adjust cells to 20-50 * 106/ml for typical staining reactions.
4. Add the appropriate number of cells to be stained into a FACS tube or 15mL conical.
5. Block with Fc Block (2.4G2). Incubate on ice for 15 min. Spin down (10 min. @ 1500 RPM, 8°C) and dump.
6. Remember to save some cells to stain single color controls for the set-up of the machine. You must have enough unstained cells and cells stained with each color used in order to set up the machine. Preferably use brightly staining reagents that stain a high percentage of cells, such as B220 or Thy1 for mice.
7. Add antibodies to stain the cells that will be sorted; use at 1x or 0.5x of the typical concentration used to stain cells for analysis. Incubate for 20-30 min on ice. Fill tube with media up to top. Spin 10 min. @ 1500 RPM, 8°C, remove supernatant and resuspend pellet.
8. Stain with secondary reagent, if needed, for 20 min. on ice. Wash as before.

9. Wash once more with Sorting Buffer. Cells must be in low protein buffer (low FCS or BSA) to prevent the sorters from clogging.
10. Resuspend cells at a concentration of 20-50x10^6/ml. This will ensure a sorting speed of approximately 18,000-20,000 events per second at the optimal pressure. Filter again through 70uM filter, again to prevent clogging.
11. Collection tubes can be blocked with FCS prior to adding collection medium. Collection medium can be supplemented with additional serum to offset the sheath buffer that will dilute the collection medium as cells are collected. Optimal conditions need to be worked out individually depending on the cells sorted, etc. Cells can be collected in either FACS tubes or 15 mL conical tubes.