SPLENOCYTE HARVEST

1. Sacrifice by cervical dislocation. Lay on right side. Soak with 70% Alcohol.
2. Make incision along contour of rib cage. Thru peritoneum.
3. Excise spleen and place into cold PBS.
4. Separate splenocytes thru 70 uM cell strainer. Rinse w **cold** PBS/ 5%FBS.
5. Spin 1400 rpm ~ 8 minutes. Remove supernatant.
6. Resuspend w **cold** PBS/ 5%FBS. Centrifuge. Remove supernatant.
7. Resuspend pellet in 5 mL **cold** ACK lysing buffer for 30 seconds.
8. Dilute ACK with ~ 45 mL **cold** PBS/ 5%FBS at exactly 30 seconds.
9. Centrifuge 1400 rpm ~ 8 minutes. Remove supernatant.
10. Resuspend in 10 mL **cold** MACS buffer.
11. Separate splenocytes thru 2nd 100 uM cell strainer.
12. Count cells. (average # from 4 quads x dilution x 10^4 x volume)
13. Centrifuge 1400 rpm ~ 8 minutes. Remove supernatant.
14. Follow appropriate MACS protocol for buffer/antibody amounts.

MAGNETIC BEAD SEPARATION

1. Soak stand and magnets with 70% Alcohol. Allow to air dry.
2. Place appropriate column in magnetic field.
   MS – 2 x 10^8 total cells LS -- 2 x 10^9 total cells
3. Prime column with 3 mL **cold** MACS buffer.
4. Apply cell suspension to column.
5. Collect cells. Wash column 3-5 times with **cold** MACS buffer.
6. Remove column from field and plunge w **cold** MACS buffer.
7. Centrifuge 1400 rpm ~ 8 minutes. Remove supernatant.

CFSE LABELING

1. Resuspend cell suspension in 3 mL **RT** PBS (w/o FCS!!) in 15 mL conical tube.
2. Add 3 uL of 5mM CFSE stock to second conical tube with 3 mL **RT** PBS.
3. Combine tubes and rock for 2 minutes at RT.
4. Neutralize CFSE by adding 6 mL **cold** FCS into same conical.
5. Count cells. (average # from 4 quads x dilution x 10^4 x volume)
6. Centrifuge 1400 rpm ~ 8 minutes. Remove supernatant.