Preparation of Cultured Cells for FACS Analysis

1. Grow culture in a T-75 flask until cells are subconfluent. In general it is good to have at least 10^5 cells for each stain. More cells (>10^6 cells) make analysis easier and faster.

2. Harvest and wash the cells as follows:

   a. For adherent cells: Aspirate media from culture and replace with 4 mls PBS/10 mM EDTA. Dislodge the cells by gently banging the flask. For cells in suspension, go directly to step b.

   b. Transfer cells to a 50 ml tube and fill with PBS. Centrifuge for 5 minutes at 1200 rpm and 4°C. Aspirate off buffer. Resuspend cells in 50 ml ice cold PBS and centrifuge again for 5 minutes at 1200 rpm. Resuspend cells in approximately 5 ml of PBS.

   c. Divide the washed cells into one tube for each stain. Use tubes which are acceptable to the FACS machine being used. Rinse out the remaining cells in each of the 50 ml tubes and combine into one tube to be used as a negative control for the FACS machine. Pellet the cells by centrifugation at 1200 rpm for 5 minutes 4°C.

3. Resuspend the samples in 100 µl primary antibody (10 µg/ml of media - this concentration is optimized for monoclonal antibodies and may vary depending on the titer of the antibody used) at 4°C. Hold on ice for 20 to 30 minutes, shaking occasionally. NOTE: Do not resuspend the negative control - it can either be left in media for the incubation time or stained with an irrelevant antibody)

4. Add 200 µl of media and shake gently. Add 200 µl of serum along the side of the tube so that it flows to the bottom and forms a cushion. Spin at 1200 rpm, 4°C for 5 minutes to pellet the cells and separate them from the free antibody. Carefully aspirate off the liquid.

5. Resuspend the cells (including the control tube) in FITC labeled secondary antibody (1/20 dilution in medium). Hold on ice for 20 to 30 minutes, shaking occasionally.

6. As in step 4, add medium and serum to form a serum cushion on the bottom of the tube. Centrifuge as before to pellet the cells.

7. Resuspend cells in 1 ml of ice cold PBS and vortex vigorously. Hold on ice, covered with aluminium foil to keep dark until analysis.