

## **STAINING FOR FLOW CYTOMETRY**

Materials: Flow Buffer (PBS / 2% FBS)

1. If using adherent cells, you may trypsinize, scrape, or use Cellstripper (Mediatech Cellgro, Cat# 25-056-C1), to get single cell suspension. For cells in suspension, harvest by centrifugation.
  - a) Centrifuge cells at 100 x g at 4°C for 5 minutes.
2. At least 10<sup>6</sup> cells will be needed for each sample (i.e. 7 x 10<sup>6</sup> for 7 samples). Transfer the required number of cells to a fresh 50 ml centrifuge tube. Bring the tube to 50 ml with Flow Buffer and invert several times. Centrifuge cells as in step 1.a.
3. Re-suspend pellet in 200  $\mu$ l/million cells with Flow Buffer. Aliquot 200  $\mu$ l for each sample into 1.7 ml microcentrifuge tubes.
4. Add primary antibody at desired concentration to appropriate tubes. Incubate for 1 hour at 4°C.
5. Wash each sample with 1 ml Flow Buffer. Centrifuge for 3 minutes at 100 x g in microcentrifuge. Aspirate the supernatant. Repeat for total of 2 washes.
6. Re-suspend samples in 200  $\mu$ l of Flow Buffer.
7. Add secondary antibody at desired concentration and incubate for 30 minutes at 4°C in the dark.
8. Repeat wash steps.
9. Re-suspend each sample in 200  $\mu$ l Flow Buffer and transfer to appropriate tubes for analysis.