Colby College BIOLOGY 225 Spring 2013
Prof. Hannum

Lab 7
2-color flow cytometry

Prior to lab, cells will be harvested from a mouse spleen. Red blood cells will be lysed, and the leukocytes resuspended in staining media (SM), ready for you to stain with fluorescent-labeled antibodies.

Stains available

FL	PE
anti mouse CD4-FL	anti-mouse CD3-PE
anti-mouse B220-FL	anti mouse CD8-PE
anti-mouse IgM-FL	anti-mouse CD69-PE
anti-mouse CD69-FL	

Prior to lab:

Think of a simple question you'd like to answer using two of the reagents above. (Don't worry, this can be a VERY simple question)

Question:

Two stains to be used (remember, one must be FL-labeled, and the other PE-labeled):

2-Color Flow Cytometry

Three important things to keep in mind while staining cells for flow cytometric analysis:

a. keep the cells on ice.

b. keep stained cells in the dark (under foil) when possible so fluorescent molecules on the antibodies don't fade. The foil can go over the top of the whole ice bucket.

c. be sure that the final prep is a single-cell suspension, or you risk clogging the intake tube on the flow cytometer. We'll filter the cells through a 100 μm mesh today to ensure this.

Procedure

Mouse leukocyte suspensions were prepared from spleens prior to class.

1. place 100 μl staining media (SM) in a labeled FACS tube
2. add 2 μl of each of the two appropriate fluorescent antibodies to this SM, making sure that the 2 μl of stain is actually delivered into the SM (not stuck on the interior wall of the tube)
    * do not use the same pipet tip for the 2 Abs – use a NEW tip each time
3. add 100 μl spleen suspension to the stain mixture; tap tube or pipet to mix well
4. place tube on ice, covered with foil, for 20 minutes.
5. add 200 μl SM, then centrifuge 3 minutes at 300 xg.
6. remove supernatant by pipetting, tap tube to loosen pellet (pellets may appear as a solid white circle or just a filmy ring, depending on the cell numbers)
7. resuspend pellet in 400 μl SM, spin in centrifuge; repeat wash (steps 6, 7) one more time
8. resuspend in 1 ml SM
9. filter cells through 100 μm Nitex mesh square into a new FACS tube, using a glass pasteur pipette. Keep covered on ice until your turn to use the flow cytometer.
10. analyze on flow cytometer

* we will hold a lottery during the staining procedure to determine the order in which the samples will be analyzed