How do I arrange to run samples at the Flow Cytometry Core Facility?

You will first need to establish an account in iLab with the facility. A **Project Based Biosafety Form** must be completed and signed by the PI prior to the submission of samples.

Will I be charged for the entire time I sign up for?

No. You are not charged for finishing early, however your time starts from the time you signed up for. We ask that you do not overbook your time as this prevents others from scheduling during that time. If you are unsure of how much time you will need contact the core facility for advice (243-2711). If you cancel with less than 24 hours notice you will be charged a minimum 1 hour time.

How many cells should I have?

There is no minimum but a good starting point is 1x10e6 cells/ml with a minimum volume of 300ul. Fewer cells mean longer collection time and potentially more background noise.

What should I bring my samples in?

Samples should be brought in 12x75mm round bottom style Falcon tubes (catalog# 352008). Note: same size tubes by other manufacturers may not fit the sample o-ring on the instrument.

Do I have to fix my cells? If so, what should I fix my cells in?

Any samples which contain potentially biohazardous material should be fixed. Cells should be fixed in a final concentration of 1-2% formaldehyde in PBS. ***Preparation of 2% Formaldehyde Solution***.

What controls do I need?

For proper instrument set up and to insure reliable results, the proper controls are needed every time an experiment is done. Unstained cells, along with positive and negative controls, are needed. Positive controls are used for compensation of fluorochrome emission overlap. Therefore positive controls must have an adequate signal and percent positive to adjust settings.

Positive controls do not have to be specific for the same marker used in the experiment. Negative controls should consist of unstained samples, isotypes, and fluorescence minus one (FMO) fluorochrome staining. Isotypes should be of the same subclass, species and fluorochrome, as the antibodies used in the experiment. The isotypes should also be used at the same concentration and F:P ratio as the specific antibody and purchased from the same manufacturer. FMO staining should include positive staining minus one of the fluorochromes. The following **template** is a basic guideline to follow.

Why do I need an Isotype control?

An isotype-matched control that has no specificity to any component of your cells provides some idea of the amount of non-specific binding that you may get with your specific antibody. In order for this to be a reasonable comparison, the isotype-matched control should be: a) at the same concentration as the specific antibody b) conjugated with the same fluorochrome at the same F:P (fluorochrome:protein) ratio and c) the same species and isotype as your specific antibody. Isotype controls are only useful as a gross estimate of non-specific binding of your specific antibody and should not be used as an indicator of where to set positive markers. There are many factors which affect the amount of nonspecific binding of an antibody, some of which an isotype control may or may not elucidate. For an in-depth discussion of the topic see Cytometry 38:78-80, 1999 (**Isotype Controls**).

How do I reduce non specific binding?

Non specific binding can be due to several reasons.

a) Too much antibody can increase the amount of non-specific binding of your negative population reducing your signal:noise. If the antibody you are using has not been titered, then a titration of your antibody should be done to determine the optimal concentration.

b) Non-specific binding can also be due to Fc-mediated binding. You can use IgG (***Lampire***) of the same species as your antibody to block non-specific binding. Incubate samples with a final concentration of 2mg/ml of IgG for 5-10 minutes at room temperature before adding specific antibodies. Using monoclonal antibodies specific for Fc Receptors to block Fc-mediated binding can also help reduce background binding. These can be purchased from various vendors (***Serotec***).

c) The use of directly conjugated antibodies can also reduce the amount of non-specific binding. If your antibody is not commercially available as a directly conjugated antibody, there are a number of simple procedures available with which you can easily conjugate your antibody. ***Molecular Probes*** offers conjugation kits for their Alexa dyes which are very simple to perform. In addition, they also offer the Zenon labeling kits which utilizes fluorochrome-conjugated Fab antibodies directed against the specific isotype of your antibody. A simple 10 minute incubation

is all that is required. Other options include conjugation kits available from ***ProZyme, Inc.*** and the standard do-it-yourself protocols (see **http://www.drmr.com/abcon**).

d) The use of a biotinylated antibody with a streptavidin fluorochrome conjugate can be a source of non-specific binding in some cells. Biotin is a component of normal cellular metabolism, and as such, there will be truckloads of it within mammalian cells. In addition, see **Molecular Probes Handbook 7.6** for a discussion of non-specific binding properties of streptavidin associated with its Arg–Tyr–Asp (RYD) tripeptide sequence which mimics the Arg–Gly–Asp (RGD) binding sequence of fibronectin.

e) Dead cells are notorious for non-specifically binding antibodies. Inclusion of a viability dye (i.e. PI, 7-AAD) in your assay will allow you to exclude the dead cell population from your analysis. You must make sure that your viability dye is compatible with the other fluorochromes in your sample. Also, cells can not be fixed when using a viability dye as this will make them permeable to the dye and all the cells will appear dead. See **viability staining protocols**. For additional tips see the **Flow Cytometry Core Laboratory at UCLA.**

What type of software do I need to analyze my data?

You will need to have software which can read FCS files. There are several different programs available for data analysis. The core facility has several software packages available on analysis workstations for data analysis. In addition you will find other options under **Resources**.