How to Design Your Experiment

Planning your experiment is the majority of the work in flow cytometry, but once you have crafted a well-thought out design, you can use the same protocol repeatedly and modify it to suit your needs. Here are the steps to follow when developing an experiment:

1) Determine what parameter or parameters you want to measure.
2) Identify antibodies that will allow you to observe those parameters.
   • Several manufacturers are available to supply you with reagents. Most antibodies can be found in multiple conjugations and more are being created all the time.
3) Ensure your markers will work in the instrument and work together.
   • Make sure that the markers you want to use will be excited by the lasers installed on our instruments:
     - Astrios Cell Sorter – 488 nm, 635 nm, 561 nm, 355 nm
     - CyAn Cell Analyzer – 488 nm, 642 nm, 405 nm
   • Look at the emission spectra of the markers and be sure that the instrument you want to use can measure that wavelength. If you are in doubt, please contact the Sort Facility Manager at fuchsj@musc.edu for technical assistance.
   • Finally, make sure that the emission spectra of all reagents you will be using in the same tube are not the same or very close to each other.
     - For instance, if you have a sample tube you want to treat with two antibodies that emit at 525 nm, the detectors will not be able to tell them apart and we will see them all as one population. You will either have to find another conjugation of this antibody that emits at a different wavelength or run the two antibodies in separate sample tubes.
     - The farther away the emission spectra of antibodies in the same tube are, the better.
     - Please note that PE and FITC have some spectral overlap. If at all possible, try not to run these in the same sample tube.
4) Allow time for your antibodies to be shipped to you. Don’t start culturing cells that will be ready in two weeks and then find you your antibody won’t arrive for six weeks.
5) **Titrate your antibodies** to eliminate background, avoid saturation, and conserve antibody.
   • See the related page on this site for instructions on how this is done.
   • Note: You will need to titrate using cells that are the same or similar to the sample you want are going to run in order to find the correct amount to use.
6) Figure out how many cells you will need for all parts of the experiment.
• Determine the number of cells you want in the final analysis to ensure that your data have statistical significance. Remember that you lose cells with each wash, decantation, and during other steps in experimentation. If you want 500,000 cells to be analyzed, do not start your labeling with 500,000 cells. Overestimation is encouraged.

• Plan to have cells for your controls in addition to the number of cells you want for your experiment. You will need at least **50,000 cells for each** of the following controls:
  - Unstained cells to measure autofluorescence.
  - Isotype controls to measure non-specificity of the antibody.
  - Compensation controls for each reagent to determine spectral overlap of emission spectra. (This is only required if you are using multiple reagents in the same tube.)

7) Plan the timeline of your experiment and the steps you will take to treat your sample.

• Read all of the manufacturer’s directions for any reagents you plan to use. Some experiments require overnight incubation, incubation at high or low temperature, or a number of other conditions. Take all incubation periods, spins, washings, etc. into account when determining how long your experiment will take to prepare and when to schedule your time on the flow cytometer.

• Ensure you have all the necessary equipment, reagents, and supplies to run the experiment before starting.

• **Filter your sample** before starting the process of immunolabeling it. This ensures minimal aggregation and a homogenous environment.

• Be sure that if your cells will have a short lifespan after being treated (i.e., not fixed), you are scheduled to run them on the flow cytometer within the timeframe that they are still viable.

• **Filter your sample** before bringing them to the flow cytometry facility or bring supplies to filter them at the facility (if possible) to minimize the chances of aggregates clogging the machine resulting in loss of your sample.

8) Schedule a time to meet with the Flow Cytometry Facility manager to discuss your experiment well in advance of the time you want to run it.

• **Bring a hardcopy of any relevant IBC registrations and a completed Flow Cytometry form** (downloadable from the website) to this meeting or we will not be able to run your cells.

• Bring a good idea of what you want to accomplish, how you are going to conduct the experiment, and (if possible) the manufacturer’s data sheets for any reagents you are going to use. We’ll be happy to work with you to refine any points that might provide challenges in your experiment.

9) Schedule your time on the flow cytometer well in advance of the time you plan to run your experiment.

10) Make it all work!