Antibody Titration Protocol

(from Dr. Mario Roederer)

Note that this information is for *any* and all antibodies--conjugate or unconjugated. Always titrate your antibodies, even commercial antibodies. Do NOT rely on what your manufacturer's instructions state--not because they are wrong, but because the manufacturer did NOT titrate the antibody under the same conditions that you are using!

Antibody titrations are NOT expressed as antibody mass per number of cells. The relevant value is antibody *concentration*, i.e., µg per mL. The number of cells that is stained is nearly irrelevant; you will find the same titration behavior whether you stain 100,000 or 1 million cells.

Generally, start titrations at about 10 ug per ml, and do 8 2-fold dilutions. Assuming staining in 100 µl (generally), start by putting 2 µg in 100 µl of the first well, then remove 50 µl and add it to 50 µl of diluent in the second well; take 50 of that and add to 50 of the third well, etc. down the line. Then come back and add 50 µl of cells to each well.

Note two different concentrations that you might use: one is the "saturating" concentration (the lowest concentration which gives you nearly maximal fluorescence), and the other is the "separating" concentration. This latter is a subjective decision on which concentration works the "best"--gives you good separation, low background. For some antibodies, this might be much less than saturating. However, you should know that at less than the saturating concentration, your final staining intensity will be time and temperature dependent.

Titrate every reagent under the conditions you plan to use it. Titrations will be temperature, time, and condition-dependent. You can have a different titration value for fix/perm protocols than you do for simple surface staining; different titration for human vs. monkey cells.

If you plan to stain 100 million cells, do NOT INCREASE the antibody by 100-fold. Generally increase it 2-5 fold, but even 1x will often be enough. If you routinely stain 100 million cells (for a sort, for example), it might be useful to do a quick, 2- or 3-point titration on that many cells. Likewise, if you stain only 50,000 cells, do NOT DECRASE the antibody amount. Remember, the relevant antibody amount to use is CONCENTRATION, expressed as amount of antibody per staining volume.

This does mean that if you titrate in 100 µl and stain in 1 ml, you will need to use 10x the antibody. Likewise, if you titrate in 200 µl and stain in 50 µl, you can use 1/4th the antibody.

The antibody titer you choose will depend on your staining time. There is no reason you can't choose a 15 min incubation for staining; it simply requires somewhat higher antibody concentration than 30 or 60 min. In fact, you could choose a concentration that works well at 3 min if you so wish!