

Titration Antibodies:

Okay, just so we are all determining optimal antibody concentrations in the same way here is a general outline.

Let's all stain 1×10^6 cells (of the population the antibody will be used with most often) in 50 μL of staining buffer. Incubate for 15-20 minutes followed by two washes if using a plate to stain or one wash of more than 1 mL if using tubes. Please consider that is important to use a cell population that has equal or greater the number of positive cells that the antibody will be used on i.e. don't titrate B220 on thymus and activation markers on cells that don't express very much CD69 if you will be using CD69 on activated T cell clones the rest of the time.

As far as a titration, obviously the specific antibody and the source will determine how much antibody will be needed. However, at bare minimum please do the following dilutions:

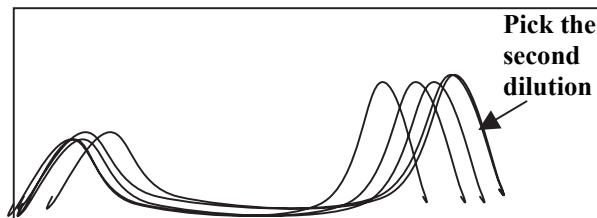
Caltag: 4 μL /sample, 2, 1, 0.5, 0.25, 0.125, 0.0625 μL /sample which can also be thought of as 1:25, 1:50, 1:100, 1:200, 1:400, 1:800, 1:1600

BD/Pharmingen: 2, 1, 0.5, 0.25, 0.125, 0.0625, 0.03 μL /sample which can also be thought of as 1:50, 1:100, 1:200, 1:400, 1:800, 1:1600, 1:3200.

Others: wing it the best you can.

Secondary reagents: Please use a nice strong antibody for the primary such as CD4 on spleen or lymph node and then titrate as above.

As far as determining the best concentration to use, most antibodies have a clear plateau for maximal staining (and will even lose signal above that, a good reason to titrate your antibodies to get maximum shift in addition to saving the lab big bucks!!). A few antibodies will not plateau and will just give a brighter signal no matter how much you use. In general, pick the concentration/volume of antibody that is one dilution before the drop in signal from the plateau.



If you are going to titrate a given antibody, please do all colors of that antibody. This will allow us to comment on which reagent worked better.

Please post your findings in the antibody database under comments including both the optimal dilution/optimal volume in μL to use. Include any comments you might have regarding how good the antibody is...i.e. gives three log shift, works better than the FITC version.