Antibody Titration

When immunolabeling a sample in preparation for running cells through a flow cytometer, steps must be taken to ensure the data acquired are accurate. One of the most important steps is titration of the antibodies you have selected. Antibodies have a range in which they bind to antigens. If too little antibody is used in the labeling, there will be an inaccurate amount of light produced by the fluorescence and, depending on the magnitude, a particle positive for the antibody may not be detected. However, if too much antibody is used, saturation occurs that masks the true amount of the target antigen in the sample. Additionally, antibodies tend to be expensive and using too much will run up your costs quickly. Therefore, we want to find an optimal concentration of antibody that approaches the saturation level, but is slightly below it. This will ensure a fluorescent signal emission that is linearly proportional to the antigen present in the sample.

To titrate the antibody, we must determine a starting concentration for where we think the proper concentration will be. Fortunately the antibody manufacturer usually takes much of the guesswork out of this equation by providing a suggested concentration to use. Please note that this is a ‘suggested’ concentration and is usually an average for several lots of the antibody whose saturation points may differ significantly. It is always a good idea to perform an antibody titration whenever you receive a supply of antibody with a different lot number than your previous supply. (To this end, it is smart to obtain large quantities from the same lot of an antibody you use often. Speaking with your antibody manufacturer’s sales representative can usually make this happen.)

Once you have an estimated starting point, create a serial halving dilution with this value in the upper portion of the dilution. For instance, if your starting point is 5μl/10^6 cells, create a serial dilution as follows:

1) Total labeling volume will be 100μl; 50μl of antibody solution (antibody and FACS buffer), 50μl of sample.
2) Label 9 5ml tubes #1 through #8 and ‘Unstained’
3) In tube #1, put 2x your highest desired concentration of antibody. In this case we will assume our highest concentration (which we believe will be beyond the saturation point) is 20μl. Therefore, put 40μl of antibody into tube #1. Now add FACS buffer to the tube to bring volume to 100μl, in this case 60μl:
   a. (Highest Concentration)x2 + Volume of FACS buffer = 100μl
4) Put 50μl of FACS buffer in the remaining tubes #2 through #8 and Unstained.
5) Pipette mix tube #1 and pipette 50μl from tube #1 to tube #2. Pipette mix.
6) Change pipette tips to prevent cross-contamination.
7) Pipette 50μl from tube #2 to tube #3. Pipette mix.
8) Change pipette tips to prevent cross-contamination.
9) Continue this process for the remaining numbered tubes. Discard the last 50μl from tube #8 after pipette mixing so that its volume is 50μl. When finished you will have 9 tubes with the following concentrations:

<table>
<thead>
<tr>
<th>Tube #</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>Unstained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration/50μl</td>
<td>20</td>
<td>10</td>
<td>5</td>
<td>2.5</td>
<td>1.25</td>
<td>0.625</td>
<td>0.3125</td>
<td>0.1562</td>
<td>0.0</td>
</tr>
</tbody>
</table>
10) Add 50ul of sample to each tube and incubate/wash in accordance with the manufacturer’s documentation. Be sure to treat the Unstained tube the same as the tubes with antibody in them (washes, spins, etc.).

11) Analyze the samples via flow cytometry to determine the concentration at which the sample is near, but not above, the saturation point.