CHILDREN'S MEDICAL CENTER
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TIPS TO GET GREAT DATA

Titrate antibodies.

Antibody titration is a quick experiment designed to find the optimal staining concentration for the antibody being used on the cells of interest. Titrating your antibodies will go a long way toward achieving good-quality flow cytometry data. Titrations help minimize noise due to nonspecific binding of the antibodies to low-affinity targets and maximize the signal of the positive particles without unnecessarily wasting antibody. This should appeal to you in multiple ways: better data, fewer experiments and saving money.

A properly tittered antibody will allow you to achieve accurate measurements of fluorescence. Some noise is unavoidable, such as that which comes from the cells (auto fluorescence), and the inherent noise in the system.

Become a fan for the FMO controls.

Setting proper gates is critical for good cytometry. One of the critical controls for doing this is the fluorescence minus one (or FMO) control. The FMO control allows for the visualization of the spread of the data due to the other fluorochromes in the panel. It is critical for sensitive measurements and rare events.

Optimize instrument voltages.

If embarking on a long-term project using the same staining panel, it is worth the time to ensure that the instrument is giving you the best sensitivity for the cells. Use the BD application setting on DiVa for this purpose (ask flow staff for more detail and help).

Value the viability dye.

Dead cells will nonspecifically take up all antibodies in the solution and can appear to be positive cells, thus confounding the results and counts. To combat this, add a viability dye. From cell impermeant dyes such as PI, DAPI and 7AAD, to the amine reactive dyes, the myriad choices will fit your assay conditions and your instrument configuration. Combating nonspecific binding is a never-ending battle that viability dyes can help with.

Adopt the median.

There are three main ways to report the central tendency of a population (median, mean or

mode). For fluorescent flow cytometry data, adopt the median. The median is the most robust measure of the central tendency on flow data for several major reasons:

- The median does not require you to know all the values, so if some are off scale, you can still calculate a median.
- The median does not assume the data fits a specific model (i.e., the normal or Gaussian distribution).
- The median is resistant to outliers.