Antibody Titration

(adapted from Mario Roederer)

Always titrate your antibodies, even commercial antibodies. The manufacturer did NOT titrate the antibody under the same conditions that you are using!

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**.

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

- Suggestion: start titrations at about 10 µg per mL, and do 8 2-fold dilutions. When staining in 100 µL start by putting 1 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 µL of that and add to 50 µL of the third well, etc. Then add 50 µL of cells to each well. If no concentration-dependent change in fluorescence intensity is detectable additional dilution steps may be necessary (For commercial antibodies with unknown concentration, start with a 1:10 dilution as the highest concentration.)

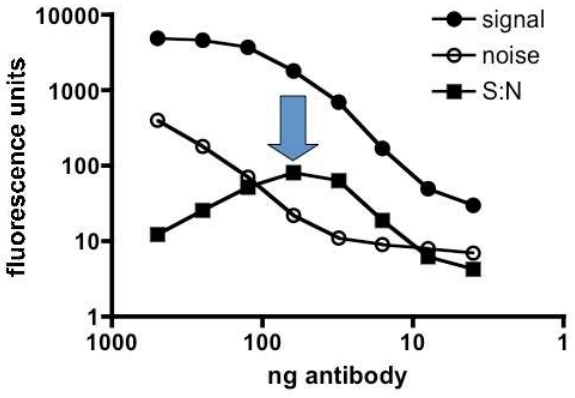
- Include unstained cells. Be aware that different cell types may have different autofluorescences and a different behaviour regarding unspecific binding of antibodies, i.e. monocytes and T lymphocytes

- For the analysis, use a scatter gate and, when staining live cells, gate out dead cells.

- Gate on the negative population and on the positive population and indicate **MFI** for each. Calculate the dilution-dependent ratio of MFI of positive (signal) and negative (noise) cells. The highest ratio indicates best **signal to noise ratio** and should be used in your experiment.

- Indicate which cells you used and your staining conditions (temperature, time, fixation conditions if applicable).

Example:



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