How to titrate monoclonal antibodies

General considerations

-Perform the titration in a cell type or stimulation condition in which a maximum amount of antigen is available for staining.

-Always check the data sheet of the antibody; it contains important information regarding antibody isotype, clone, host, reactivity, expression pattern, working dilution, sensitivity to fixation/permeabilization steps and (sometimes) the staining profile made by the producer company.

-For surface staining: use staining buffer: PBS 1X + 2% BSA + 2 mM EDTA or PBS 1X + 2% FCS + 2 mM EDTA .

-Intracellular staining: Different conditions and reagents for fixation and permeabilization might be evaluated.

-If possible, stain $1x10^6$ cells in 100µl of antibody dilution.

-When titrating antibodies that require a secondary antibody, determine first the best concentration of the primary antibody and then evaluate the secondary. Test different concentrations of the primary antibody, keeping a fix concentration of the secondary (try initially the one recommended by the producer, usually 1/200). Once you establish the best titer for the primary, test different concentrations of the secondary.

-The best antibody concentration is that which produces the best discrimination between the positive and negative cells. Accordingly, the most important measurement is the fluorescence intensity of staining (signal) vs. nonspecific binding (noise), the **signal-to-noise ratio**. Using an antibody at a very high concentration, might give a very strong positive signal, however it will give a strong nonspecific binding as well.

Considerations for intracellular staining:

The protein concentration inside cells is orders of magnitude higher than membrane protein, thereby exacerbating nonspecific binding (NSB). Because it is desirable to resolve specific binding from NSB, it is necessary to systematically evaluate both. Isotype controls provide an estimate of the NSB component, whereas specific antibody binding is composed of both specific and NSB components.

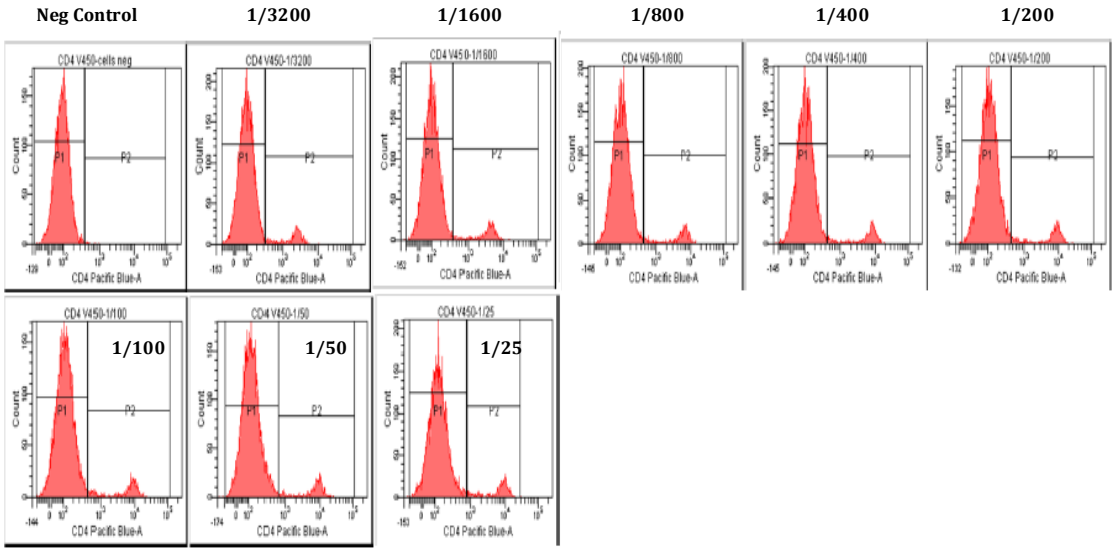
When using a secondary antibody, prepare tubes containing the second antibody alone to distinguish between NSB due to Ig, (revealed by the isotype control), and that produced by the second antibody itself.

Protocol

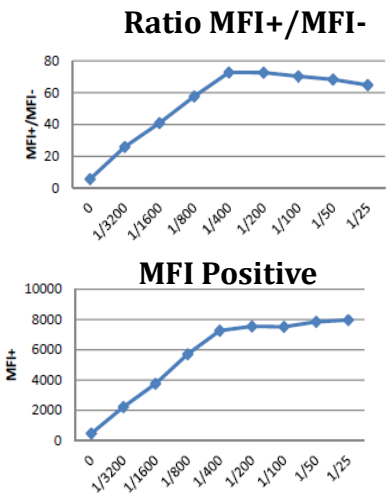
1. Perform 8 serial dilutions from 1/25 down to 3200 per antibody: Dilute 8µl of stock mAb in 192µl of staining buffer. This results in a 1/25 dilution. Take 100µl of this and dilute into 100µl of staining buffer, perform serial dilutions until 3200 dilution. You can do it within a 96 well plate.
2. Incubate 20 minutes on ice protected from light with foil, spin and remove the supernatant
3. Wash the cells with 200µl of staining buffer
4. Resuspend in 200µl of PBS 1X.
5. Acquire the samples starting with the ones stained with the most diluted antibody concentration.

Analysis

Express the results in a histogram per dilution starting with the most diluted. Make a region in the negative and positive populations and calculate the mean fluorescence intensity (MFI) of each and the ration between both.



Dilution	MFI Positive	MFI Negative	Ratio
0	471	84	5.6
1/3200	2226	86	25.8
1/1600	3759	92	40.85
1/800	5709	99	57.66
1/400	7274	100	72.74
1/200	7551	104	72.60
1/100	7520	107	70.28
1/50	7851	115	68.26
1/25	7968	123	64.78



Make a graph with the ratio and a graph with the MFI of the positive population. The second graph will show the concentration at which saturation is occurring. **In this case the dilution 1/400 seems to be the appropriate.**

References

Current Protocols in Cytometry (1997) 4.1.1-4.1.13 Copyright © 1997 by John Wiley & Sons, Inc. Contributed by Carleton C. Stewart and Sigrid J. Stewart