Titering Directly Conjugated Antibodies to Extracellular Antigens

(from Current Protocols in Cytometry (1997) , C.C. Stewart & S.J. Stewart)

It is always important to titer your antibodies. This includes antibodies from commercial sources.. The process is simple and usual requires less than 10 tubes. Perform the titering on the flow cytometer that you would normally use to analyze or sort your samples.

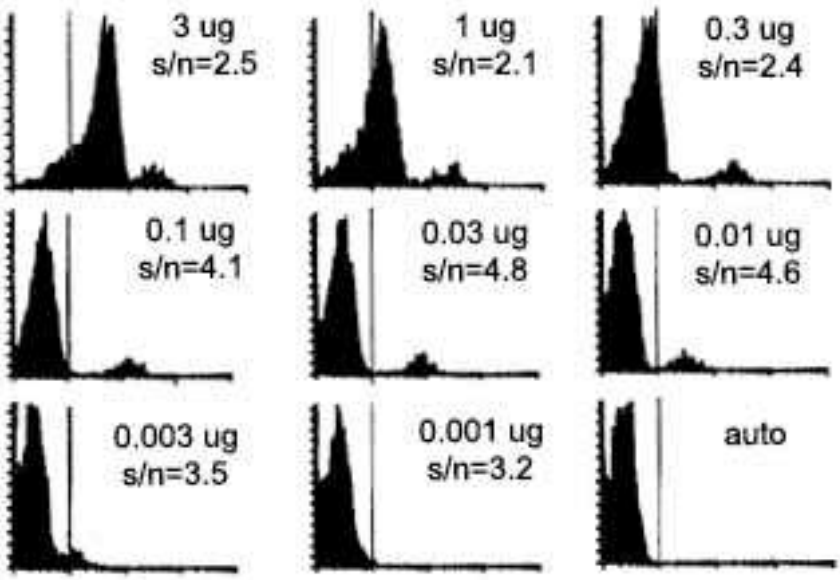
Material:

1. Stock solution of specific, fluorochrome-conjugated antibody to be titered
2. Target cells
3. PBS without calcium or magnesium
4. 2% ultrapure formaldehyde in PBS

Procedure:

1. Determine the concentration of specific antibody conjugate in the stock solution and centrifuge 10 min at 15,000 X g, 4°C. Leave aggregated antibody in pellet
2. Prepare 30 μl containing 9 μg antibody (300 μg/ml) in PBS Prepare six 1/3 serial dilutions (10 μl to 30 μl) in PBS
3. Prepare target cell suspension containing 5-10 X 10^6 cells/ml.
4. Add 10 μl of each antibody dilution to 50-μl aliquots of cell suspension in separate 12 X 75-mm polypropylene test tubes. Also prepare a control tube containing only cell suspension. Incubate 15 min at 4°C.
5. Centrifuge 3 min at 1500 x g. 4°C.
6. Remove supernatant and resuspend cells in residual solution.
7. Add 200 μl of 2% ultrapure formaldehyde in PBS
8. Acquire and analyze samples on cytometer.
    - Adjust markers using control sample, so that <1% of events are above the marker
    - Acquire Mean Channel Fluorescence values for both the positive
    - (signal) and negative (noise) cell populations for each dilution..
    - Compute the signal to noise ratio by dividing the MCF value for positive cells by that for negative cells. Plot these values as a function of antibody dilution.
    - The optimal titer is the one that generates the highest ratio, because this provides the greatest discrimination between positive and negative cells.

In the example below, 0.03 μg is the optimal antibody concentration.


3 ug
s/n=2.5
1 ug
s/n=2.1
0.3 ug
s/n=2.4
0.1 ug
s/n=4.1
0.03 ug
s/n=4.8
0.01 ug
s/n=4.6
0.003 ug
s/n=3.5
0.001 ug
s/n=3.2
auto