Flow Post-its
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Antibody Titration for Flow Cytometry

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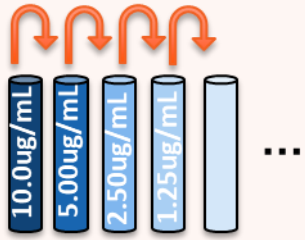
Antibody Titration is one of the most important steps in Panel optimization to ensure **best Resolution**. It allows you to find the **optimal concentration** of antibody that results in the **brightest signal of the positive population** while **avoiding background** staining, thus maximizing **signal-to-noise ratio**. Additionally, it helps **save money and reagents.**

Sample Staining Tips

1. Titrate your antibodies under the **same conditions as your experiment** (same cell type, temperature, incubation time, total volume, same number of cells)
2. You must include **Viability Dye** for reliable data due to non-specific binding of antibody to dead cells
3. Adding **FC block** is critical when doing titration to prevent FC mediated binding
4. If your marker of interest is expressed at low levels, adding another marker may help you resolve the populations (e.g., CD45 can be added in an heterogeneous tissue to pull out leukocytes)
5. Start your Ab concentration at 10 µg/mL and do 8 serial 2-fold dilutions

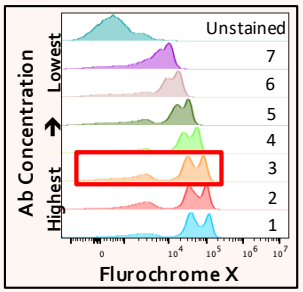


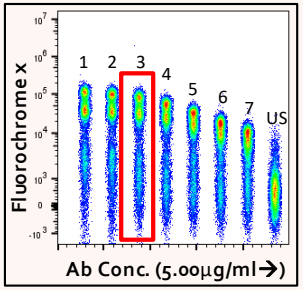
Analysis Tips

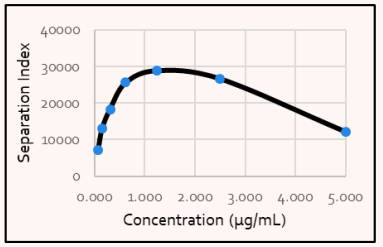
1. Record at least 1,000 live cells expressing your marker of interest
2. Gating strategy: Live cells ➔ FSC/SSC ➔ Singlets ➔ Positive and Negative populations
3. Positive and Negative **gates need to be adjusted** for each sample since the populations will shift
   Note: population percentages should be approximately the same.
4. Visual analysis of your data alone should never be used to determine the optimal concentration
5. Calculate the **Separation Index (SI)** for each condition using the MFI of the negative and positive populations
   Choose optimal antibody concentration where the SI is maximized. This can be adjusted to a lower concentration to allow for reduced spread in your multicolor experiments).

$$Separation\ Index = \frac{MedianPositive - MedianNegative}{(84\%Negative - MedianNegative)/0.995}$$

Telford et. al (2009), doi.org/10.1002/cyto.a.20790







Flow Cytometry
Core Facility

https://fccf.mskcc.org

Memorial Sloan Kettering Cancer Center