NOTE TECHNIQUE

CICC N°002 – v001

ANTIBODY TITRATION

Why titrate antibodies ?
1- To **OPTIMIZE SEPARATION** of positive and negative populations
2- To contribute to results **CONSISTENCY AND ROBUSTNESS**
3- To **SAVE MONEY**.

General recommandations:
- ❑ Titrate the antibody under the conditions you will be using them.
- ❑ Set up the PMT voltages so that the negative and positive population fall in the linearity range of the detector.
- ❑ Test 2-3 concentrations around the data sheet recommandation.

« Saturating » concentration	« Separating » concentration
++ for markers where expression is indicative of function or identity.	OK for markers whose expression is ON/ OFF and are used as identity markers
✓ Antigen density per cell measurable ✓ Cell number should not impact results.	Level of antigen expression cannot be quantified.

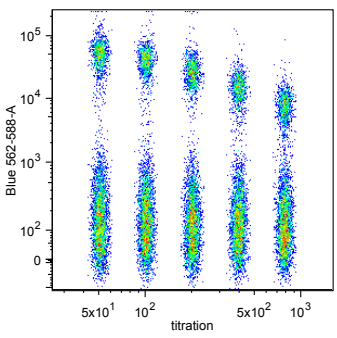
1- On **HOMOGENEOUS POPULATION** (clear pos/neg peaks), calculate the stain index

$$SI=(Med_P-Med_N)/ 2*SD_N$$

Or alternatively, the Signal/ noise ratio

$$S/N=Med_P / Med_N$$

2- For populations with **HIGH VARIANCE** in fluoresence intensity, graph the MFI vs Ab concentration.



What is the optimal concentration ?

- ✓ Maximum stain index of signal/ noise ratio.
- ✓ Point where MFI starts to plateau.
- ✓ Point where background fluorescence is comparable to unstained sample.

+ Download the Excel file for a complete tutorial and ready to use worksheets.