

MODE OPERATOIRE / OPERATING PROCEDURE	VERSION
BASIC ANTIBODY TITRATION PROTOCOL	A

Reagents and Materials

- Cells to be stained
- FACS buffer – can be cell dependent
 - o 1x Ca/Mg²⁺ free PBS
 - o Either FBS (1-10%) or BSA (0.1-1%)
 - o EDTA (0.5-5mM) if you have very sticky cells
 - o Commonly used: 1% FBS in 1X PBS (consider filtering FBS to remove debris)
- Antibodies
- Eppendorf or FACS tubes

Procedure

1. Prepare single cell suspension (lyse RBCs if needed)
2. Count cells
3. Adjust cell concentration to 1-5 million cells per 50 μ L FACS buffer
 - a. Try to stain the same number of cells you plan to use for your final experiment
4. Add Fc block to the pooled cells following the manufacturers protocol
 - a. Usually 1-5 μ l per 100 μ l at 4°C for 10-15 minutes or more
5. Make 4 tubes per antibody (1.5 ml Eppendorf or 5 ml FACS tubes) **serial dilutions**
 - a. Tube 1 = 0.3 μ g **dilution 1/50**
 - b. Tube 2 = 0.1 μ g **dilution 1/100**
 - c. Tube 3 = 0.03 μ g **dilution 1/400**
 - d. Tube 4 = 0.01 μ g **dilution 1/800**
 - e. You may choose to go higher (3 μ g, 1 μ g) or lower (0.003 μ g, 0.001 μ g) depending on the antibody **dilution 1/1600 if necessary**
 - f. Beware: some antibodies are 0.5 mg/ml and others are 0.2 mg/ml. Brilliant violet antibodies tend to be odd concentrations. Make sure you check the concentration!
 - g. If it is a small population, you may need other antibodies to gate the population
 - h. You should have a population with both a positive peak and a negative peak
 - i. Other cells can be used if you are struggling to find a clear positive and negative population, but it is ideal to titrate on your specific cells of interest
6. Pipette antibodies to have the appropriate μ g in 50 μ l FACS buffer
 - a. Prepare these either in advance (antibodies can be saved in FACS buffer for several weeks) or while cells are in Fc block
 - b. **IMPORTANT:** vortex and quickly spin down the antibody tubes before pipetting to avoid unusual highly fluorescent debris during acquisition on the cytometer
7. After the Fc block incubation, pipette 50 μ L of cells into antibody-containing tubes for staining
 - a. 50 μ l of cells + 50 μ l antibody mixture = 100 μ l total volume
8. Incubate for 30 minutes in the dark at 4°C (cover tubes in alu foil if needed)
9. Wash cells with FACS buffer
 - a. 1 ml if staining in Eppendorf tubes
 - b. 2 ml if staining in flow tubes
10. Centrifuge



MODE OPERATOIRE / OPERATING PROCEDURE	VERSION
BASIC ANTIBODY TITRATION PROTOCOL	A

11. Aspirate, pipette, or dump tubes to remove supernatant
 - a. Be careful not to disturb the pellet!
12. Resuspend in FACS buffer
 - a. Volume depends on the number of cells
 - b. At least 350 μ l for acquisition on the Fortessa or Symphony

