Extra and Intracellular Staining

Done in 96 well plates with 1-5x$10^{6\ cells}$/well

Extracellular Staining

- Dilute the Antibody in 25 ul of Facs WASH, add to the well
- Shake and incubate 10-15 min on ice
- Wash with Fasc Wash (3 drops) 2 times. Spin (2 min at 1200rpm) and flick
- Add the secondary antibody, shake, incubate and wash. Spin and flick.

Intracellular Staining

- Fix cells: resuspend pelleted cells in 200ul of 1% PFA. Let sit RT 20min (it can be on ice) Spin and flick
- Wash cells: resuspend in 200ul of Perm Buffer. Spin and flick.
- Stain with the first Antibody (1:100) in 100ul Perm Buffer for 30 min on ice.
- Wash cells: resuspend in 200ul of Perm Buffer. Spin and flick.
- Wash cells: resuspend in 200ul of Stain Buffer. Spin and flick.
- Wash cells: resuspend in 200ul of Stain Buffer. Spin and flick.
- Resuspend pellet in 200ul of Stain Buffer and analyze by FACS

Solutions

Staining Buffer

2% FCS, 0.2 % NaN_3 in PBS

Permeabilization Buffer

Stain Buffer + 0.1% Saponin