Intracellular staining for intra-cytoplasmic antigens

Reagents

30X PBS-A
120.0g NaCl
3.0g KCL
17.25g Na_2HPO_4
3.0g KH_2PO_4
Dissolve in 400mL boiling distilled H_2O
Cool to RT
Test pH of 1X sample, adjust 30X accordingly to 7.2-7.4
QS to 500mL with dH_2O

4% Formaldehyde in Hypertonic PBS-A
14mL 10X PBS
40mL 10% formaldehyde
46mL dH_2O

PBS/BSA/Azide
PBS
0.5% BSA
0.1% sodium azide
adjust buffer pH to 7.4-7.6, store at 4°C

PBS/BSA/Saponin
PBS
0.5% BSA
0.1% sodium azide
0.1% saponin
adjust buffer pH to 7.4-7.6, store at 4°C

Extracellular Staining

1. Resuspend 1-10E6 cells in 1 mL in staining medium
2. Pipette 100mcl of cell suspension per well (96 well polystyrene round-bottom, nonsterile, Rainin). Centrifuge plate for 10 min at 700 RPM (80xg).
3. Decant supernatant by gently flicking plate over sink and blotting on a paper towel. This will leave on average 10-15mcl residual volume/well.
4. Add antibodies to well. Most preparations work well at 1-2 mcl/well. **The critical factor is the antibody concentration, not the number of cells/well or the absolute amount of antibody/well.** Total volume must be kept below 20mcl/well.
5. Cover plate with aluminum foil. Incubate 30 min at 4°C.
6. Wash 1X: add 200mcl/well of PBS-A medium and centrifuge plate for 10 min at 700 RPM. Decant supernatant.

Intracellular Staining

Formaldehyde steps

1. Add 100mcl PBS-A, then 100mcl of 4% formaldehyde in hypertonic PBS-A.
2. Let stand at RT for 20 min.
3. Wash once in PBS/0.5%BSA/Azide

Saponin Steps

1. Add 150mcl per well PBS/0.5%/BSA/Saponin. Mix samples well. Incubate 10 min RT.
2. Centrifuge plate for 10 min at 700 RPM. Decant supernatant (spin and flick).
3. Add cytokine antibodies (most preparations work well at 1-2 mcl/well).
4. Mix well, incubate 30 min RT.
5. Wash x1 PBS/0.5%BSA/Saponin
6. Wash x1 PBS/0.5%BSA/Azide
7. Resuspend in 200mcl of staining medium and acquire data.

Adapted from procedures, complements of Donnenberg Lab, University of Pittsburgh