Direct Immunofluorescent Staining of Cell Surface Antigens

Reagents:

- Antibodies
- FcBlock
- PBS
- Staining Buffer (PBS + 3% FBS + 0.01% Sodium Azide)

Direct Staining in 12 x 75 mm tubes:

- Collect cells and transfer approximately 1 x 10^5 - 10^7 cells/mL into a 5mL (12 x 75 mm tube
- Wash 2 x with ice cold PBS, by centrifuging at 1000 rpm, 10 mins, 4 degrees in a centrifuge with a swinging bucket rotor.
- Remove supernatant
    - Avoid disturbing the pellet during aspiration and take care not to aspirate to dryness
- Disrupt the pellets by flicking the tubes.
- Block surface Fc receptors for 10 minutes at room temperature (or 15-20 minutes at 4 degrees) – DO NOT WASH

Table 1: Fc Receptor Block Reagents

Cell Type	Fc Block Source Reagents
Human	Polyclonal human IgG 10% normal human serum diluted into PBS
Mouse	Purified antibodies directed against FcγII/III receptor
Rat	Purified antibodies against FcγIII receptor
Note: Most FCM Antibody reagent companies sell blocking reagents	

- Stain cells with directly conjugated antibodies at optimized concentrations (see antibody titration) for 15 – 20 minutes at RT or 30 minutes at 4 degrees, in the dark.
    - Prepare single stain compensation controls for each fluorochrome you intend to analyze (beads or cells)
    - Include an unstained sample
    - Prepare fluorescent minus one gating control samples
    - Prepare matched isotype controls for each antibody-fluorophore combination (if necessary)
- Wash cells 2x with PBS and resuspend in staining buffer.
- Add a dead cell marker (PI or 7-AAD), incubate 10-15 minutes at RT (do not wash)
    - Choice of marker depends on panel.
- Analyze cells by flow cytometry as soon as possible
    - If you cannot analyze the cells within an hour of staining, consider fixing with 2%PFA (see protocol). This will preserve the samples for up to one week.
    - Do not add 7-AAD or PI to samples that require fixation. A different choice of dead cell marker is required for fixed cells.

Direct Staining in 96-well microtitre plates:

- Collect cells and transfer approximately 1 x 10^5 – 1.5 x 10^6 cells to each assay well.
    - Add less cells/well when working with cultured cell lines

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- Wash 2 x with ice cold PBS, by centrifuging at 1000 rpm, 10 mins, 4 degrees in a centrifuge with a swinging bucket rotor.
- Remove supernatant with a multi-channel pipette and disrupt the pellets by pipetting.
    - Avoid disturbing the pellet during aspiration and take care not to aspirate to dryness
- Continue as above for the remaining steps.