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 $1 \times 10^5$ - $10^7$ 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>
<thead>
<tr>
<th>Table 1: Fc Receptor Block Reagents</th>
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<tbody>
<tr>
<td>Cell Type</td>
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<tr>
<td>Human</td>
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<tr>
<td>Mouse</td>
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<td>Rat</td>
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</tbody>
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- 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 $1 \times 10^5$ – $1.5 \times 10^6$ 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.