

## Flow Cytometry Protocol for Intracellular Staining

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### 1. Solutions and Reagents

- 1.1. 1X PBS
- 1.2. Blocking buffer: 0.5% BSA in 1X PBS
- 1.3. 2% paraformaldehyde (1% solution - optional for storing samples)
- 1.4. 1X FACS permeabilizing solution (BD Biosciences cat. #340973)
- 1.5. Fluorescently-conjugated secondary antibody (various forms)

### 2. Protocol

- 2.1. Collect  $1 \times 10^6$  cells/sample.
- 2.2. Wash cells once with blocking buffer.
- 2.3. Fix cells with 2% paraformaldehyde and incubate at room temperature for 10 min.
- 2.4. Wash cells once with blocking buffer.
- 2.5. Add 0.5 ml 1X FACS permeabilizing solution and incubate at room temperature for 10 min.
- 2.6. Wash cells once with blocking buffer.
- 2.7. Incubate cells in blocking buffer for 30 min at room temperature.
- 2.8. Add primary antibody at the appropriate dilution and incubate for 30 min at room temperature.
- 2.9. Wash twice with blocking buffer and incubate with fluorescently-conjugated secondary antibody for 30 min at room temperature.
- 2.10. Wash cells twice with blocking buffer.
- 2.11. Re-suspend cells in 1X PBS and analyze on flow cytometry. Samples can be kept in 1% paraformaldehyde at 4 °C overnight.