FLOW CYTOMETRY STAINING

Charles Yin
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Bowdish Lab, McMaster University
Hamilton, ON, Canada
www.bowdish.ca

EQUIPMENT AND MATERIALS

- Equipment:
  - Centrifuge set at 4°C
  - Bucket with ice
  - PBS
- Materials:
  - Antibodies for flow cytometry
  - FACS wash (0.5% BSA, 5mM EDTA in PBS)
  - FACS block (1% BSA, 5% FBS in FACS wash)
  - 4% paraformaldehyde (PFA) in PBS

PROTOCOL

1. Wash cells with cold PBS.
2. Lift cells and re-suspend in 1ml cold PBS.
   a. Cells should not be exposed to trypsin nor scraped off the plate. Use cell lifters where possible.
   b. Keep cells cold on ice at all points after this step to prevent membrane turnover.
3. Pellet cells by centrifuging at 4°C at 2,000rpm for 10min.
4. Re-suspend pellet in 100µl FACS block.
5. Incubate on ice for 1h.
6. Add primary and secondary (or conjugated) antibodies.
   a. Most protocols recommend adding primary and secondary antibodies separately. I have seen no difference either way. Adding both antibodies together reduces the total time of this protocol.
   b. Keep cells away from light as much as possible after this step to avoid photobleaching of fluorophores attached to the antibodies.
7. Incubate on ice for 1h.
8. Wash 3× with 1ml cold PBS.
9. If cells are to be analyzed immediately, re-suspend cells in 200ul cold PBS. Otherwise, re-suspend cells in 200ul of cold 4% PFA and store up to 24h at 4°C away from light.
10. Strain cells into flow cytometry tubes prior to analysis. Keep tubes covered when not in use.