FACS Analysis Protocol (Ariel/Eric)

Complete stimulation (if necessary) and detach cells using scrape/EDTA method

1. Wash media away
2. Add 10uM EDTA (in PBS) …incubate 10min. and scrape
3. Add to 15 mL falcon tube and spin cells @ 3000 rpm for 1 min.
4. Transfer cells (in 100uL FACS buffer) to 96 well plate

Add primary antibody, which includes isotype control and Ab against protein of interest.

- Typically use 5ug/mL of primary antibody
- Mix cells with pipettor
- Cover w/ clear 96 well adhesive cover
- Wrap in aluminum foil (foil not necessary if Ab is unconjugated)
- Incubate 30min. on ice (if cells are permeabilized consider room temperature)
- Spin cells @ 3000rpm for 1min.
- "Smack" plate quickly on paper towel to remove buffer

Add secondary antibody at a 1:400 concentration…100uL per well.

- Incubate 30min. w/ foil & adhesive cover on ice
- Add samples to FACS tubes and bring up to 200uL total volume in FACS buffer

FACS buffer

1X HBSS, 0.1% BSA, 0.02% NaAzide