This protocol was optimized for the detection of intracellular IL-6 in mouse mast cells (both MC/9 and primary BMMC) from *in vitro* culture, using a PE-conjugated antibody. It is based on the protocol developed by BioLegend.

Different cells, different tissues, different proteins, and different antibodies should be optimized carefully. For example, you will need ACK Lysis Buffer if analyzing PBMCs from blood.

Materials:

- FC buffer (0.1% BSA in 1X PBS, or purchased from BioLegend #420201, note that their formulation includes azide!)
- Monensin
- Fixation buffer (4% paraformaldehyde; or BioLegend #420801)
- Permeabilization Buffer (this is basically FC buffer with 0.1% saponin; BioLegend #421002)
- Antibody with directly conjugated fluorophore.
- 2mL microcentrifuge tubes
- Standard range of micropipettes with tips

Things people mess up that are easily prevented (so pay close attention!):

- Double check your dilutions. For example, the monensin and permeabilization buffers are both concentrated stock solutions.
- Watch your pipette tips – be gentle when aspirating! You should use 2mL tubes (not 1.7mL!) with the conical bottom because the cells will pellet on the hinge side where the straight part of the tube meets the cone. Therefore, you can more easily aspirate liquid from the bottom of the tube. IF YOU DISRUPT THE CELL PELLET.-9.1 ( ) Etention!):