

General Immunocytochemistry Used by Isakson Lab:

- 1) Add room temperature 2% PFA in PBS for 10 minutes; slow shaker
 - a. Or use -20 °C, 50/50 acetone/methanol for 3 minutes
- 2) Wash 2X with PBS, pH 7.4, 5 min each; medium shaker
- 3) Add blocking solution at room temperature for 30 minutes; slow shaker:
- 4) Add primary antibody in blocking solution; place on slow shaker at 4°C overnight
- 5) Wash 2X with PBS, pH 7.4, 5 min each; medium shaker
- 6) Add blocking solution at room temperature for 30 minutes; slow shaker
- 7) Add secondary antibody in blocking solution at room temperature for 2 hours; show shaker; **KEEP COVERED FROM LIGHT** (usually 1.5 X primary concentration; e.g., primary 1:500, secondary 1:750)
- 8) Wash 2X with PBS, pH 7.4, 5 min each; medium shaker
- 9) Mount w/ DAPI or SYTOX to visualize nuclei (optional)
- 10) View under microscope