CB: 8/6/03

Immunocytochemistry

Prep: -Warm several hundred mls of 1X PBS to 37°C
-Make 1% PFA in PBS or 3% formaldahyde in PBS (500uL/ well)
-Make 5% goat serum in PBS

1. Wash cells 2X with warm PBS (1ml in a 12 well plate); wash only 4 wells at a time to avoid drying and add PBS gently down the side of the well to avoid knocking cells off.
2. Fix cells in PFA for 20 min or formaldehyde for 1 min. (10-15 min to permeabilize cells
3. Wash cells in PBS 2X for 5-10 min.
4. Block in 5% normal goat serum (NGS) for 20 min to overnight in 4°C (add 0.3% triton X to permeabilize cells).
5. Transfer the cover slips to parafilm and add 100-150uL of primary antibody in blocking buffer. Usually between a 1:100 and a 1:1000 dilution.
6. Leave for 1-2 hour at RT or overnight in 4°C.
7. Wash 3X with PBS for 10 min each.
8. Add 2° antibody (if needed) in blocking buffer at a dilution of 1:100 to 1:1000 (usually more dilute than primary).
9. Leave for 30 min-2 hours at RT
10. Wash 3X with PBS for 10 min each.
11. Wash backside of cover slip with dd H2O (not cell side!) to rinse away salts.
12. Place cover slip face down onto microscope slide with a drop (about 20uL) of vectasheild. (Vectasheild may contain a nuclear stain for counter staining: Topro (1:1000-1:2500), Dapi, etc.).