Immuno-Cytochemistry / Immunofluorescence

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REFERENCES:

1. BD Cytofix/Cytoperm™ (cat # 554714)
2. http://en.wikipedia.org/wiki/Saponin

Materials:

1. Serotec Cytoperm Kit or Pharmingen Cytofix/Cytoperm Kit (Fisher/BD cat# 554714). Note: we add goat serum to Permwash (10%), but this isn't absolutely necessary.

   OR
2. Homemade solutions:
   a. Homemade Permeabilization wash (permwash): (0.5% Saponin in PBS, 10% Goat serum); filter using 0.2µm filter; store @4°C.
   b. Homemade Permfix: 4% PFA (diluted to 4% with Homemade Permeabilization wash); store @4°C

3. 4% Paraformaldehyde (diluted from 16% paraformaldehyde; EMS#15710)
4. (optional) 0.1M glycine in Permwash, pH 7.4 (one hour at room temp after fixation step)
5. PAP Pen (sigma #Z377821)
6. Fluoromount G (Southern Biothech, VWR#100241-874)
7. coverslips

Instructions:

- Remove frozen tissue sections from -80°C freezer and immediately immerse in 4%PFA/PBS (r.t.) for 5 min.
- After the incubation, dry the area around the section (using a combination of wiping and aspiration), and encircle with PAP pen. (do this quickly –don't let the tissue section dry out!!).
- Cover section with Permfix solution (~100ul –enough to cover the section).
  - Incubate for 5 minutes @ room temp
  - Remove fixative by vacuum
  - Rinse with 1x Permwash, then aspirate.
- Gently wash cells again with 1X Permwash solution. Let sit for 3 min, and repeat.
- Block in Perm/Wash for 10-15 min.
- Dilute 1° Ab in 1X Permwash solution (optimal dilution depends on the specific Ab, but we typically start with 1:100).
  - Incubate for 1 hr @ 37°C (Alternatively, incubate @4°C overnight. For some proteins, (low abundant or nuclear, e.g., ERalpha), incubate for 1-2 days at 4°C, or use higher concentration antibody.
- After incubation, wash the tissue sections with 1X Permwash solution (3 x 5 min)
- Dilute 2° Ab in 1X Permwash solution (use 1:400 dilution of Alexafluor Ab)
  - Add enough to cover the tissue/cells and incubate for 1 hr @ room temp. Although not absolutely required, we often place the slides on a rocking platform on a low setting.
- After incubation, wash cells with Permwash (2 x 5 min)
- Stain nuclei with DAPI or ToPro3, if needed.
- Rinse several times with **PBS**. Mount coverslip with Fluormount-G.

NOTE: Because of Saponin's reversible nature on cells and its ability to permeabilize cells without destroying cell morphology, it is used in laboratory applications to treat cells in order to facilitate peptide or reagents such as antibodies to enter cells instead of the harsher detergent triton X-100. It is also done on whole cell preparations such as cell smears and cytospins where the cell membrane is intact. To preserve the permeabilizing effect, saponin has to be used in all processes involved in the staining steps or otherwise removed after reagent of interest has reached the cell. (Wikipedia)