IMMUNOHISTOCHEMISTRY

Immunocytochemistry (ICC, IHC) by definition is the demonstration of a tissue constituent in situ by detecting specific antibody-antigen interactions where the antibody has been tagged with a visible label. The visual marker may be a fluorescent dye, colloidal metal, radioactive marker or more commonly, an enzyme for light microscopy. Experimental samples ranging from frozen sections, cell culture/suspension, to whole tissue samples have been used. Ideally, maximal signal strength along with minimal background or non-specific staining are required to give optimal antigen demonstration.

Fixation:

Fixatives are needed to preserve cells and tissues in a reproducible and life-like manner.
(An ideal fixative should: preserve the tissue and cells without any shrinking or swelling and without distorting or dissolving cellular constituents; prevent putrefaction by bacteria and prevent autolysis by cathepsin containing cells; stabilize and protect tissues and cells against the detrimental effects of subsequent processing and staining procedures. Typically, cells are fixed in 4% paraformaldehyde in phosphate buffer. Sucrose can be added to the fixative to enhance preservation of membrane structure and processes during cross-linking. Sucrose also increases the density of the fixing solution, which causes it to drop to the bottom of the wells and ensure rapid fixation. In certain cases, paraformaldehyde can mask antigenic epitopes from recognition by specific antibodies. In those cases, other fixatives, such as cold methanol, can be used.)

Comment: Paraformaldehyde (PFA) fixative is very dangerous and much care should be taken while following this procedure! PFA in powdered form is very dangerous. Wear a mask and measure in a chemical fume hood! Do not allow yourself or anyone in the lab to be exposed to the powder or fumes coming from the hot water.

Ex: **40 μm brain slices** from dissected brains post-fixed in 4% (w/v) PFA overnight (8-24 h) at 4°C with gentle shaking and washed in PBS for 3 hr x 3 times at room temperature with gentle shaking. Slices stored in anti-freezing solution at -20.

1. Wash, one quick rinse then overnight in PBS at room temperature (gentle shaking).
2. Wash 3X10 min PBS (change well or just remove PBS).

Permeabilization + Blocking (to block non-specific staining - block unspecific binding of the antibodies).
(If the target protein is expressed intracellularly, it is very important to permeabilize the cells)

3. Incubate 1hr in 0.2% Triton X-100 / 3%BSA / PBS (500μl/well – 4 slices per well MW24) at room temp with gentle shaking.
4. Rinse 5 min in PBS

Incubation:

5. Incubate in primary antibody at 4ºC for 24hr with gentle shaking (ON at 4 ºC and the rest of the day at room temp). Make up the antibody in 0.2% Triton X-100 / 3%BSA / PBS, and centrifuge the antibody mix 10 min room temp in microfuge before putting on cells. (300μl/well – 4 slices per well MW24)
6. Wash 3X10 min PBS.
7. Incubate in secondary antibody overnight at 4ºC. (Make in 0.2% Triton X-100 / PBS and centrifuge first, as for primary.)
8. Wash 3X10 min PBS.
9. Incubate with Flash Phalloidin (green/Red 1:75) for 40/50 min at room temperature. (Make in 0.2% Triton X-100 / PBS - 300μl/well)
10. Wash 3X10 min PBS.
11. Incubate with DAPI (1:10000) for 20/30 min at room temperature. (Make in 0.2% Triton X-100 / PBS - 300μl/well)
12. Wash 3X10 min PBS.
13. Mount on clean slide in a drop of mounting medium (DakoCytomation).
14. Seal coverslip with nail polish to prevent drying and movement under microscope
15. Store in dark at -20 or 4°C.

Note: Never let cells/tissue dry out.