Microscopy Imaging Center Coimbra

CENTER FOR NEUROSCIENCE AND CELL BIOLOGY

IMMUNOCYTOCHEMISTRY (CULTURED NEURONS, HELA…)

Immunocytochemistry (ICC) 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)

1. Fix 15 min room temp in 4% paraformaldehyde/4% sucrose/PBS that has been prewarmed to 37ºC. (The fix can be made ahead and stored for up to one week at 4ºC.)
2. Rinse, one quick rinse then 2X5 min in PBS.

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.

NOTE: 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.)

Permeabilization

(If the target protein is expressed intracellularly, it is very important to permeabilize the cells)

3. Incubate 5 min room temp in o.25% Triton X-100 / PBS.
4. Rinse 5 min in PBS

Blocking and Incubation:

(to block non-specific staining - block unspecific binding of the antibodies).

5. Incubate 30 min at 37ºC in 10% BSA/PBS
6. Incubate in primary antibody 4ºC 16-40hr OR 37ºC 2h. Make up the antibody in 3% BSA/PBS, and centrifuge the antibody mix 10 min room temp in microfuge before putting on cells.
7. Wash 6X2 min PBS.
8. Incubate in secondary antibody 37ºC 45 min. (Make in 3% BSA and centrifuge first, as for primary.)
9. Wash 6X2 min PBS.
10. Dip quickly in water and mount on clean slide in a drop of mounting medium.
11. Seal coverslip with nail polish to prevent drying and movement under microscope
12. Store in dark at -20 or 4°C.

Note: Never let cells dry out.