Trinity College Dublin
Coláiste na Tríonóide, Baile Átha Cliath
The University of Dublin

Standard Operating Procedure

Immunocytochemistry

Original Author: Sarah McComish

mccomiss@tcd.ie

PI: Prof Maeve Caldwell

Original Date: 21/03/2021

Revision Date:

Description

Immunocytochemistry (ICC) is a laboratory technique widely used to anatomically visualise the specific protein or antigen localisation within cells. A protein specific primary antibody is used to bind to the protein of interest. A secondary antibody specific to the species of the primary antibody is then used to apply a colour via a conjugated fluorophore. The now immune-labelled protein may be visualised using the appropriate microscope. A fluorescent microscope is required to visualised fluorescently labelled proteins while a standard light microscope may be used to visualise proteins labelled with DAB, which forms a visible brown precipitate in its oxidised form.

Procedure

Sample preparation

1. Fix cells in 4% paraformaldehyde (PFA; 25 min, RT).
2. Wash to remove PFA (3 x 10 min washes) with PBS.
3. Add PBS to wells to prevent cells from drying out.
4. Seal plate with parafilm and store in fridge until staining.

Immunostaining protocol

1. Block cells with 10% normal goat serum (NGS) in Triton X-100 (0.3% in PBS; PTx; Sigma-Aldrich) for 2 h.

2. Stain with primary antibodies against the neuronal/astrocytic antigens of interest in NGS (2%) and PTx (0.06%) at 4ºC overnight

   [A negative control without primary antibody is used as a control to reveal any non-specific staining.]

3. Wash with wash buffer (0.02% PTx; 3 x 10 min washes).
4. Incubate with species-specific secondary antibodies in PTx (0.02%) for 2 h in the dark.
5. Wash with wash buffer (3 x 10 min washes).
6. Incubate cells in Hoechst (1:1000 in PBS, 30 min, RT; Cell Signalling Technology, USA) to counterstain the nucleus.
7. Wash once with wash buffer to remove Hoechst.
8. Add PBS (500 µL) to keep the cells hydrated until mounting.
9. Mount coverslips on slides in mounting media (5 µL; 1:1 PBS-glycerol), seal with clear nail varnish and stored at 4ºC in the dark.

Image acquisition

Cells are imaged using a fluorescent microscope (Carl Zeiss Axio Imager Z1 microscope, x20-x40 objectives) with attached camera (AxioCam HRm; Carl Zeiss, Germany). All images must be taken at the same magnification and exposure times while collecting images for each experiment. Cells may be counted manually using the ImageJ (ImageJ 1.50i) counting tool, or densitometry carried out by measuring the level of fluorescence of each cell. All analysis must be performed on unmodified images.

Risk Assessment

Triton-X 100 is toxic to wildlife therefore liquid waste contaminated with Triton must be collected and disposed of via Hazmat.

Useful Resources