M. Fero — 3/13/98

Immunofluorescence of Cultured Cells

Materials
L-lysine coated glass cover slips or charged glass slides
Neutral Buffered Formalin (Sigma HT50-128)
0.5% NP40 in PBS
(2.5 N HCl or 0.07 N NaOH for BrDU staining only)
Primary and secondary antibodies
Vectashield (Vector Labs)

Protocol

1) Grow cells on L-lysine coated glass slips or cytospin cells onto charged glass slides.

2) Fix cells for 5 min. in neutral buffered formalin.

3) Permeabilize the nucleus by incubating in 0.5% NP40 in PBS at r.t.

4) Rinse in 3 changes of PBS for a total of 10 minutes.

5) For BrDU staining denature the DNA by one of the following:

a) soak in 2.5N HCl at 37°C for 15 min, or

b) 0.07N NaOH for 2 min at room temp

5) Add 100 µL primary antibody (titer determined emperically ~10x the concentration used in a western). Cover with a glass slip and place in a humidified chamber at r.t. for 1 hr.

6) Float the coverslip off by dipping into a jar of PBS, and rinse as in 4).

7) Add secondary antibody as in 5) and 6) above. (e.g. FITC-conj goat anti-rabbit (1:1000) or Biotinylated isotype specific anti mouse for immunoperoxidase staining). Wash as before.

8) To minimize quenching of flourochrome mount with Vectashield (Vector labs) and cover with a glass slip.