

Immunocytochemistry

Materials

- Mounting Medium : PVA 20g + dH₂O 30ml → 80°C 2-3 days → Tris pH 9.0 5ml + DABCO 2.5g + Glycerol 5ml
- 10% paraformaldehyde / PBS (+ Triton X-100 0.25% v/v)
- 30% H₂O₂ (Don't touch without globe)

Transfection

Fixation

- Fix the cell dishes in 10% paraformaldehyde in PBS for 15 min at room temperature

Permeabilization

- incubate the samples for 10 min with PBS containing 0.25% Triton X-100.
- To block endogenous peroxidase, incubate cells with 0.2% peroxide(H₂O₂) / PBS for 5min.

Blocking

- Incubate cells with 1% BSA in PBS for 30 min to block unspecific antibodies.
- Incubate cells in the diluted antibody in 1% BSA in PBS for 1 hour

Development

- Prepare DAB solution
DAB 0.2mg/ml (5mg DAB tablet / 25ml of 50mM Tris-HCl , pH=7.5) + 0.015% H₂O₂
- Incubate cells for 30-60 sec. at RT
- Counterstain with hematoxilin
- Mount with hydrophobic mounting medium.