Basic immunofluorescence of resin sections

Materials

Phosphate buffered saline, pH 7.4 (PBS) Bovine serum albumen, fraction V (BSA) Tween 20 Primary and secondary antibodies Humid chamber

Method

- 1. Dry 1µm sections onto poly-l-lysine coated slides using a 37°C hot plate.
- 2. Rinse with PBS-T pH 7.4 (0.1% v/v Tween 20)
- 3. Add blocking solution (5% (w/v) BSA in PBS-T). Incubate for 30-60min at room temperature in a humid chamber.
- 4. Add primary antibody, diluted in 1% BSA in PBS-T (the dilution required is very variable, from 1 in 5 to 1 in 100). Incubate for 2hrs at room temperature or overnight at 4°C in a humid chamber
- 5. Wash sections with PBS-T, with several changes over 20min.
- Add secondary antibody (with fluorescent tag), diluted in 1% BSA in PBS-T T (the dilution required is again very variable, from 1 in 100 to 1 in 1000).
 Incubate in the dark for 1hr at room temperature in a humid chamber.
- 7. Wash slides 2x with PBS-T, 2x with PBS and 3x with dh₂O.
- 8. Observe under fluorescence.