

## **Immunohistochemistry: Immunoperoxidase staining for frozen tissue sections**

- 1/ Leave frozen tissue sections warm at room temperature for 1 to 2 hours.
- 2/ Fix the sections in cold acetone for 10 min.
- 3/ Wash twice in PBS.
- 4/ If necessary, block endogenous peroxidase by incubating in 0.1% H<sub>2</sub>O<sub>2</sub> in 70% methanol for 10-30 minutes.
- 5/ Wash once in PBS.
- 6/ Incubate sections for 1 hour in 1.5% normal blocking serum in PBS, derived from the same species in which secondary has been raised. Remove blocking serum from slides.
- 7/ Incubate with appropriately diluted primary antibody for at least 1 hour at room temperature or overnight at 4°C. Wash twice in PBS.
- 8/ Incubate the sections with the peroxidase conjugated secondary antibody at recommended dilution. Incubate for 30-60 minutes at room temperature. Wash twice in PBS.
- 9/ Stain with the appropriate substrate DAB solution or AEC solution for 10 minutes.
- 10/ Wash with demineralised water.
- 11/ Briefly counterstain with heamatoxilin for 1-10 minutes.
- 12/ Wash gently in running water until blue colour is clearly visible.
- 13/ Dehydrate by increasing solution of ethanol and xylene solvent, mount with medium and examine by light microscopy.