

IMMUNOLOCALIZATION OF PROTEINS (HRP)

1. Dewax slides 2 x 10' in histoclear.
2. Rehydrate by passing through the following ethanol series (2' each):
99% ethanol
99% ethanol
95% ethanol
85% ethanol
3. Quench endogenous peroxidase by incubating in 3% H₂O₂ in methanol for 15' at room temperature.
4. Continue rehydrating:
70% ethanol
50% ethanol
30% ethanol
dH₂O
dH₂O
5. Incubate in **PBS/BSA** for 5' at room temperature.
6. Drain slides, place in a large petri dish containing wet tissues around the side. Put 50-100 µl 0.1% Goat IgG in **PBS/BSA** on each slide.
Cover
petri dish and incubate for 15' at room temperature.
7. Drain slides and rinse in **PBS/BSA**.
8. Drain slides, place back in petri dish and put 50-100 µl 1° antibody on each (1/10 - 1/100 dilution in **PBS/BSA**). Incubate for 15' at room temperature.
9. Wash 2 x 15' in **PBS/BSA**.
10. Drain slides, place back in petri dish and put 50-100 µl biotinylated

2° antibody on each (1/10 - 1/100 dilution in **PBS/BSA**). Incubate for 15' at room temperature.

11. Wash 2 x 15' in **PBS/BSA**.

12. Drain slides, place back in petri dish and put 50-100 µl biotinylated/streptavidin/horseradish peroxidase complex on each (1/10 - 1/100 dilution in **PBS/BSA**) (Amersham). Incubate for 15' at room temperature.

13. Wash 2 x 15' in **PBS/BSA**.

14. Drain slides and add 100 µl **stain** to each. Watch reaction under microscope. When signal is clear, rinse slides with dH₂O and dehydrate back through the ethanol series (2' each).

15. Wash 2 x 5' in histoclear, add a drop of Permount/DPX mountant and place a coverslip over sections.

PBS/BSA (1L)

0.15M NaCl	30 ml 5 M
10mM phosphate buffer pH 7.0	10 ml 1 M
1mgml ⁻¹ BSA	2 g

Stain

500 µl 1mgml⁻¹ **DAB** (diaminobenzoic acid)
499 µl H₂O
50 µl 8% NiCl
1 µl 30% H₂O₂ (add last immediately before use)

DAB is extremely carcinogenic but can be deactivated by bleach. Therefore, buy powder in sealed vials which can be injected with liquid to make the 1mgml⁻¹ stock. Aliquot in 500 µl lots and store at -20°C. The solution should be clear. - if it is not you will get high background. Anything that comes into contact with DAB should be soaked in bleach overnight before disposing to the drains.