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
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   - 95% ethanol
   - 85% ethanol

3. Quench endogenous peroxidase by incubating in 3% \( \text{H}_2\text{O}_2 \) in methanol for 15' at room temperature.

4. Continue rehydrating:
   - 70% ethanol
   - 50% ethanol
   - 30% ethanol
   - \( \text{dH}_2\text{O} \)
   - \( \text{dH}_2\text{O} \)

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 \( \mu l \) 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 \( \mu l \) 1\(^{\circ}\) 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 \( \mu l \) 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.