P.A.P.

Day I

Prepare Slides:

- Cut sections @ 3 microns on clear glass slide.
- Place in 58-60 degree C. incubator overnight

Day II

- Place 200 ml 1/10 PBS in a 37 degree C. incubator.
- Weigh out 0.2 gm trypsin.
- Make up albuminized 1/10 PBS:
  - 1 ml bovine albumin per 100 ml 1/10 PBS (This solution is used to prepare all antibody dilutions and for washing slides).

Procedure

(1) Transfer slides directly from 58-60 degree C. incubator to:

1. xylene ..... 10 min.
2. xylene ..... 10 min.
3. xylene ..... 10 min.
4. xylene ..... 10 min.
5. 95% alcohol ..... 5 min.
6. Cold Tap H_2O ..... 5-10 min.
7. 37 degree Tap H_2O ..... 3-5 min.

(2) Add 0.2 gm trypsin to 200 ml 1/10 PBS in 37 degree C. incubator.

(3) Transfer slides to trypsin solution for 6 minutes at 37 degrees C. (Discard after use).

(4) Rinse in cold tap H_2O for 5 min.

Inhibition of Endogenous Peroxidase

(5) Transfer slides to freshly prepared 200 ml of methanol + 2 ml 30% H_2O_2 for 30 minutes

(6) Rinse in cold tap water for 5 minutes

(7) 95% alcohol ..... 5 min.

(8) Abs. alcohol ..... 5 min.

(9) Chloroform ..... 5 min.

(10) Acetone ..... 5 min.

(11) Cold Tap H_2O ..... 5 min.

(12) Albuminized PBS ..... 5 min. minimum

Primary Antibody

(13) Prepare dilutions of primary antibody using albuminized PBS 140 - 200 ul per slide.

(14) Prepare slides one by one by drying outside and leaving a film of the albuminized PBS around the section (NO AIR BUBBLES). (DO NOT LET THE SECTIONS DRY!).

(15) Cover section with diluted primary antibody (one slide at a time) for 30 minutes in a humidification chamber.

(16) Wash each slide carefully with 1/10 PBS (albuminized) using a squeeze bottle.

(17) Transfer to a staining dish of the albuminized PBS.

Secondary Antibody

(18) Prepare dilution as follows:
for 20 slides
PBS 1/10 - 2700 ul
Normal Human - 300 ul
Serum

Shake well and discard 200 ul of this solution.
Add 200 ul peroxidase conjugated rabbit immunoglobulines to mouse immunoglobulins. Mix well.

(19) Prepare slides as for primary antibodies and add secondary antibodies for 30 minutes.

(20) Wash with albuminized PBS and return to staining dish of PBS.

Teritary Antibody

(21) Prepare dilutions as for secondary antibody using peroxidase conjugated swine immunoglobulins to rabbit immunoglobulins.

(22) Prepare slides as before and add antibody for 30 minutes.

(23) Wash well with PBS and return to staining dish of PBS.

Revelation of Peroxidase Staining:

(24) Prepare DAB:

Dissolve 6 mg of DAB (diaminobenzidine) for 6 slides in 10 ml Tris-HCL (0.05M). Add 3 ul of 30% H_2O_2 just before use. Keep in dark. (If larger quantities are needed, use several tubes ready for H_2O_2.

10 ml 10 ml 10 ml

(25) Arrange slides in humidification chamber. Put DAB on each slide for 3 to 5 minutes. (Do not attempt to reveal too many slides at one time - maximum 15 slides).

(26) Rinse with old tap H_2O to stop reaction.

(27) Return to a staining jar of water.

Counterstain

(28) Haematoxylin for 10 to 20 seconds

(29) Rinse in cold tap H_2O.

(30) 95% alcohol

(31) Absolute alcohol

(32) Acetone

(33) Xylene

Mount with Permount