7/2/03

# Immunoperoxidase Staining
# Cytospins

- Prepare cytospins
- Fix for 10 minutes in a 50:50 mixture of room temperature acetone:methanol..
- Air –dry for 10 minutes
- Label the slides with the Antibody and Species
- Use a Pap-Pen to draw a containment circle around the sample.

  The circle should be as small as possible without touching the cells.

PRIMARY ANTIBODY

- Vortex dilutions of primary antibodies (Abs, prepared in PBS+BSA).

  Use 30-50µl – just enough to cover the cells.
- Place slides in a moist chamber filled with paper towels soaked in water.
- Incubate slides in the moisture chamber for 1 hour at room temperature. Timing is not critical – can use ± 15 minutes with no problem.
- Blot off the excess Ab and soak slides in FA buffer 1 X for 5 minutes.

BIOTYNILATED ANTIBODY (Vectastain – blue bottle; species-specific):

10 µl Ab

2 ml PBS+BSA

- Cover each section with 1-2 drops of biotynilated Ab and incubate at room temperature for 30 min, in a moisture chamber.
- Blot off the excess Ab and soak slides in FA buffer 1 X for 5 minutes.

ABC REAGENT (Vectastain – orange bottles, not species-specific):

20 µl reagent A

20 µl reagent B

2.0 ml of PBS+BSA

- Place 2 drops on each slide and incubate for 30 minutes at room temperature in a moisture chamber.
- Blot off the excess ABC solution and soak slides in FA buffer 1 X for 5 minutes.

AEC COLOR DEVELOPMENT:

0.014 g AEC (Sigma, A5754) in a small glass tube

2.5 ml of NN-dimethyl formamide (Sigma). This eats plastic – use glass pipet.

17 ml acetate buffer in a 50 ml conical tube

- Dissolve AEC in dimethyl formamide. Add AEC/dimethyl formamide mixture to the acetate buffer with a Pasteur pipette (***Be sure it's clear. Discard and start over if precipitant forms***). Add 10 µl of cold 30% hydrogen peroxide and invert to mix.
- Cover the slides generously and incubate for 10 minutes at room temperature
- Blot off the excess solution and soak slides in FA buffer 2X for 5 minutes each.
- Stain the slides with hematoxylin for 5 min.
- Rinse 3x with $H_2O$ by dipping up and down until water runs clear
- Remove the excess of water from slides.
- Place 1-3 drops of Aquamount in the middle of the slide and drop the coverslip on top.

FA buffer for washes:

10 liters ddH2O

100 gms Difco FA buffer (purchase from VWR)

BSA solution

100ml DPBS with $Ca^{2+}$ and $Mg^{2+}$

0.1 gm bovine serum albumin

Mix and store at 4°C. Check before each use and discard if contaminated

Acetate buffer

10ml 0.2M NaAc (16.406 gm sodium acetate/liter ddH2O)

1ml 0.2M acetic acid (6ml acetic acid in 500ml ddH2O)

89ml ddH2O

check and adjust pH to 5.2

Mix and store at 4°C. Check before each use and discard if there is any evidence of contamination. The pH is critical, and it can shift over time. Discard acetate buffer that has not been recently used and prepare fresh.

Vectastain kits – purchased from Vector Laboratories.