# GENERAL IMMUNOHISTOCHEMISTRY PROTOCOL

- 1. Deparaffinize and rehydrate sections as follows:
  - 3 x 3' Xylene
  - 3 x 2' 100% Ethanol
  - 1 x 2' 95%, 80%, 70% Ethanol (each)
  - 1 x 5′ 1X PBS
- 2. Antigen retrieval methods:

### •Sodium Citrate Antigen Retrieval:

a. Place slides in a glass slide holder and fill in the rest of the rack with blank slides (10 total) to ensure even heating.

b. Place rack in 600 ml of 10 mM Sodium Citrate (pH 6.0, 100 mM stock) in a glass 2L beaker. Mark a line at the top of the liquid on the beaker.

- c. Microwave for 20 minutes total, replacing evaporated water every 10 min.
- d. Cool slides for 20 minutes in the beaker.
- e. Wash 3 x 5' in ddH<sub>2</sub>O, 1 x 5' in 1X PBS.

## Proteinase K Antigen Retrieval:

a. Make a fresh solution of:

25 ul of 20 mg/ml Proteinase K 2.5 ml of 1 M Tris-Cl, pH 8.0 0.5 ml of 0.5 M EDTA, pH 8.0 to 50 mls with  $ddH_2O$ 

b. Incubate slides in solution at 37°C for 5 min (do NOT pre-warm Prot K solution). A Coplin steining for works well for this step

solution). A Coplin staining jar works well for this step.

c. Wash 3 x 5' with 1X PBS.

## •Urea Antigen Retrieval:

- a. Make a fresh solution of 1 M urea
- b. Place slides in a glass slide holder and fill in the rest of the rack with blank slides (10 total) to ensure even heating.

c. Place rack in 600 ml of 1 M urea in a glass 2L beaker. Mark a line at the top of the liquid on the beaker.

d. Microwave for 10, 20 or 30 minutes total, replacing evaporated water every 5-10 minutes.

- e. Cool slides for 30 minutes to 1 hour in the beaker.
- f. Wash  $3 \times 5'$  in ddH<sub>2</sub>O,  $1 \times 5'$  in 1X PBS.
- 3. Block endogenous peroxidases: (optional step)
  - a. Soak slides in 90 ml methanol/10 ml 30% H<sub>2</sub>O<sub>2</sub> for 10-15 minutes at room temp.
  - b. Wash 3 x 5' with 1X PBS.

4. Shake and wipe off excess 1X PBS. Circle all sections with a PAP pen. Add 50-75 ul of blocking buffer to each section immediately, so that the sections don't dry out. Don't touch sections with tip.

#### Blocking buffers: •5% BSA/0.5% Tween-20 in 1X PBS •3% BSA in 1X PBS •3% BSA/0.1% Tween in 1X PBS •MOM (for mouse and rat monoclonal antibodies, use Molecular Probes secondary antibodies with MOM basic kit)

5. Incubate 1 hour to overnight at room temperature in a humidified chamber. Do not let the slides touch each other.

6. Dilute primary antibody in blocking buffer (dilutions will vary depending on your antibody). Add 50-75 ul per section and incubate 1 hour to overnight at room temperature in a humidified chamber.

7. Drain primary antibody off section. Wash slides  $3 \times 10^{\circ}$  in 1X PBS. (You may have to wash slides in 1X PBS + 0.1%-0.5% Tween-20 for some primary antibodies)

8. Dilute biotinylated secondary antibody 1:250 to 1:750 in blocking buffer. Add 50-75 ul per section and incubate 1 hour at room temperature in a humid chamber.

9. Drain secondary antibody and wash slides 3 x 10' in 1X PBS.

## For secondary antibodies that are peroxidase conjugated, skip to step 11.

10. Add 1 drop of ABC (Ready to Use; Vector to each section and incubate samples for 30 minutes at room temperature. Wash 3 x 5 minutes in 1X PBS

11. Make DAB according to Vector protocol in ddH<sub>2</sub>O. WEAR GLOVES.

2.5 ml ddH<sub>2</sub>O 1 drop buffer; mix 2 drops DAB; mix 1 drop H<sub>2</sub>O<sub>2</sub>, mix.

(If you want a gray-black stain, add 2 drops of the Nickel Solution; mix) Add immediately to slides and wait for color change (approximately 2-10 minutes). Drain slides and wash  $ddH_2O$  for 5 minutes (the slides can be left in water longer). Dispose of DAB waste with bleach.

## 12. Counterstain with hematoxylin:

Dip 8-12x (fast)	Hematoxalin
Rinse	deionized water
1 x 5′	<u>Tap</u> water (to allow stain to develop)
Dip 8-12x (fast)	Acid ethanol (to destain)
Rinse 2 x 1'	Tap water
Rinse 1 x 2'	Deionized water (can leave overnight at this stage)

### 13. Dehydrate sections:

3 x 5′	95% ethanol
3 x 5′	100% ethanol (blot excess ethanol before going into xylene)
3 x 15′	Xylene (slides can be left in xylene overnight to clear any remaining water)

14. Coverslip slides using Permount (xylene based).

Place a drop of Permount on the slide using a glass rod Angle the coverslip and let fall gently onto the slide. Allow the Permount to spread beneath the coverslip, covering all the tissue.

15. Dry overnight in the hood.

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## Supplies:

- PAP pen
- Mouse on Mouse (MOM) Immunodetection
- Vectastain Elite ABC Reagent R.T.U.
- DAB Substrate Kit for Peroxidase
- Permount (Histological mounting medium)

Research Products International #195505 Vector Laboratories Vector Laboratories, PK-7100 Vector Laboratories, SK-4100 Fisher Scientific #SP15-100