GENERAL IMMUNOFLUORESCENCE 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 4 x 5' in ddH₂O, 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 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 4 x 5' in ddH₂O, 1 x 5' in 1X PBS.

3. 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)
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4. Incubate 1 hour to overnight at room temperature in a humidified chamber. Do not let the slides touch each other.

5. 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.

6. Drain primary antibody off sections. 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)

7. Dilute fluorescently conjugated secondary antibody 1:750 - 1:1000 in 1X PBS. Add 50-75 ul per section and incubate 1 hour at room temperature in a humid chamber. **KEEP DARK**.

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

9. Mount the sections in 3:1 Vectashield:DAPI (Vector labs). Coverslip and seal edges with clear nail polish.

Supplies:

•PAP pen	Research Products International #195505
 Mouse on Mouse (MOM) Immunodetection kit 	Vector Laboratories
•VECTASHIELD® Mounting Medium	Vector Laboratories #H-1000
•VECTASHIELD® Mounting Medium with DAPI	Vector Laboratories #H-1200