

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 5 min.
- d. Cool slides for 20 minutes in the beaker.
- e. Wash 4 x 3' in ddH₂O, 1 x 3' 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 ddH₂O
- 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 minutes.
- e. Cool slides for 30 minutes to 1 hour in the beaker.
- f. Wash 4 x 3' in ddH₂O, 1 x 3' 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. 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)

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 humid chamber. KEEP DARK.
6. Drain primary antibody off section. Wash slides 3 x 10' in 1X PBS. You may have to wash slides in 1X PBS + 0.1%-0.5% Tween-20 for some primary antibodies.
7. Dilute 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.
8. Drain secondary antibody and wash slides 5 x 10' in 1X PBS.
9. Mount the sections in 3:1 Vectashield:DAPI (Vector labs). Coverslip and seal with clear nail polish.

Supplies:

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| •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 |