

Indirect Immunofluorescence for Paraformaldehyde-Fixed Paraffin-Embedded Tissue

I) Tissue Fixation.

1) Fix tissue in cold *fresh* **4% paraformaldehyde¹ (PFA)** in 1x PBS as soon as possible after time of death of the host.

2) Process at once or store indefinitely in 70% ETOH at 4°C.

II) Pre-treatment of sections:

1) De-wax and re-hydrate tissue sections:

Warm slides at 60° for at least 15'

Xylene 2 X 10'

100% ETOH 2 X 2'

95%, 95%, 80%, 70%; Water 1 minute each

2) Immerse slides in **10mM Citric Acid Monohydrate buffer pH 6.0** (Fisher # A104-500) (1.36 g in 650ml H₂O (for 1 rack) or 1.88g in 900ml H₂O (for 2 racks)) and incubate in microwave. The solution will come to a boil in a few minutes. Let it boil for 6 minutes. (Set our microwave for 14 min for 650 ml or 17 min for 900 ml) *Don't let the slides go dry. This step enhances the availability of the antigen (i.e., by breaking aldehyde 'cross-links' and, in the case of BrdU immunohistochemistry, denatures dsDNA and releases it from histones effectively exposing BrdU)*

3) Remove from microwave and let cool in buffer 10'

4) Rinse in gently-running tap water 10'

5) Lie slides out in hybridization box. Wash with 1X PBS 2 X 5'
(Use PAP pen to restrict subsequent volumes of reagents applied to the sections).

6) Block with **Coulter 'Protein Blocker' Reagent** at RT. 30'
DO NOT RINSE; Pour excess blocker off and add diluted Ab, as described below.

III) Primary Antibody Incubation

1) Incubate sections with **primary antibody diluted in PBT or Zymed antibody diluent**:(use water-soaked paper towels to create a moist atmosphere in the hybridization box)

O/N at 4°C

NOTE: stock is kept frozen in 10 µl aliquots (0.5mg/ml) at -80 °C. After thawing keep in at 4 °C.

2) Rinse and then wash in PBS 3X 10'

2) Secondary antibody incubation

- 1) Incubate sections with appropriate **secondary antibody -Cy3 or Cy2** (Jackson) diluted in **1:600 in PBT or Zymed Diluent** in moist chamber in the dark
2 hrs @ RT
- 2) Rinse and then wash in PBS using light-tight chambers 3 X 10'
- 3) Rinse briefly in ddH₂O
- 4) Remove excess water and let slides dry in the dark
- 5) Mount coverslip with **Kierkguard** mounting medium and seal with clear nail polish
- 6) Store slides at 4°C in a light-tight box until you are ready to look at them.

4% PFA per 50 ml:

- 1) Mix 2 g PFA (Fisher #04042), 6 L 10N NaOH, and 25 ml DEPC H₂O into 50 ml conical tube.
- 2) Heat to 60°C and place on rotator until PFA is dissolved. *Be careful not to overheat the solution.*
- 3) Chill before open the tube. Add 5 ml of 10x PBS and bring the volume up to 50 ml with DEPC H₂O .

For 50mL PBT Solution, add the following reagents in these amounts:

ddH ₂ O	43.5mL
10XPBS	5.0mL
10% BSA	0.5mL
10% Triton-X100	1.0mL

*Adjust to volume needed; store at 4°C for up to one week