Indirect Immunofluorescence for Snap-frozen OCT-Embedded Tissues

I) **NO** Tissue Fixation.
   1) Remove tissue and immediately place in OCT and snap freeze in liquid nitrogen.
   2) Place the box in the cold while sectioning (Never let the slides warm up). Store at -80°C

II) Post fixation
   1) Place slides directly into prechilled Methanol/Acetone (1:1) and fix the slides for 10 min in -20°C. Rinse in water.
   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 antibodies 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 -Cy2 or –Cy3 (Jackson) diluted 1:600 in PBT or Zymed Diluent in moist chamber in the dark
Catherine Lee, Ph.D.

2 hrs @ RT

2) Rinse and then wash in PBS using light-tight chambers 3 X 10’

3) Rinse briefly in ddH$_2$O

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 H$_2$O 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 H$_2$O.

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

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume</th>
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<tbody>
<tr>
<td>ddH$_2$O</td>
<td>43.5mL</td>
</tr>
<tr>
<td>10X PBS</td>
<td>5.0mL</td>
</tr>
<tr>
<td>10% BSA</td>
<td>0.5mL</td>
</tr>
<tr>
<td>10% Triton-X100</td>
<td>1.0mL</td>
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</tbody>
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*Adjust to volume needed; store at 4°C for up to one week*