Immunolabeling Protocol Shiv-IGB Core-010410

1. Dewax slides
   - Fresh Histoclear…….5min
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   - 100% EtOH…… …… .5min
   - 70:30 EtOH/PBS…… ……..5min
   - 50:50 EtOH/PBS…… ….5min
   - 25:75 EtOH/PBS…… ..5min
   - 100% FRESH PBS……...5min 3x
2. PAP Pen Mark
   - dry slide with kimwipe quickly avoiding direct contact with tissue
   - draw outline around the tissue with PAP pen
3. Block and Incubate
   - block with IT signal FX for 15 min/IMC chamber in dark (ask Shiv)
   - rinse in PBS for 1 second

   incubate with Primary antibody Dilute PRIMARY Ab in PBS/ and 10% Signal FX (Example: for a 10 mL of diluted PAB = 1 mL of Signal Fx + 0.1 mL of PAB + 8.9 mL PBS)-You can add another primary if the hosts are different Ex., mouse and rabbit primaries

   Incubate for 2 h, apply sufficient volume and keep the lids of incubation tray closed).
1. KEEP wells that will receive secondary antibody only samples in Signal FX until it is time to incubate with secondary antibodies
2. Wash slides 3x (5 minutes each) with 1x PBS.

(All steps from here on, keep the cell dish covered with a small box on the desk on top of the incubation tray so that they are in the dark or using an aluminum foil)

3. Dilute secondary antibody 1:200 Goat Anti-Mouse Alexa 488Ab in PBS containing 10% Signal FX the total volume of 2 mL (for example for a 10 mL of diluted SAB = 1 mL Signal Fx + 0.5 mL SAB + 8.5 mL PBS)
   a. Keep other wells with blocking buffer where secondary antibody will not be used.
   b. Incubate for 1h
      - Wash all wells 3x (5 minutes each) with 1x PBS.
4. Dilute second secondary antibody 1:200 Goat Anti-Rabbit Alexa 568Ab (if any) in PBS containing 10% Signal FX the total volume of 2 mL (for example for a 10 mL of diluted SAB = 1 mL Signal Fx + 0.5 mL SAB + 8.5 mL PBS)
   - Keep other wells with blocking buffer where secondary antibody will not be used.
   - Incubate for 1h
   - Counter stain with DAPI for nuclei 10ug/mL solution in PBS for 15 min and wash 2x 5min in PBS.
   - Rinse in PBS and dry with kimwipe
5. Mount Coverslip
   - 20-30ul of Prolong Gold onto slide
   - Use cover glass 22 x 50 or 22 x 40 #1.5
   - Roll coverslip on (flex coverslip with finger and roll the slide on from one side to the other
   - Remove excess mounting medium with kimwipe by pressing the slide gently on a fresh kimwipe while the coverslip side is on the kimwipe, do not move, apply pressure on corners only and do it for three times in new areas of the tissue paper.
   - Store in dark for 24hrs at Room Temp (typically in a drawer/cupboard)
   - Apply topcoat nailpolish on the edges of the coverglass-slide interface
6. Store

- in slide container in dark @ 4ºC refrigerator, until imaging in a confocal or fluorescence microscope. Questions, contact Shiv 333-1214 or sivaguru@illinois.edu