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JOHNS HOPKINS MEDICINE

Hackam Lab
PFA fixed tissues

1. Dewax and hydrate paraffin embedded tissue sections:
   Warm slides at 60°C for 30' in vacuum incubator
   - Histoclear 2x5'
   - 100% ETOH 2x2'
   - 95% 1x5'
   - 70% 1x5'
   - Water 1x5'
2. Immerse slides (in plastic slide rack) in 10mM Citric Acid buffer pH 6.0 (Fisher cat.# A104-500; Citric Acid Monohydrate =0.63g in 300 ml H_20).
3. Heat in microwave oven till boil (~ 2' 15")
4. Stop and reset the microwave to 6 minutes, power 3 (30%).
   Important-listen the microwave periodic turning on and off sound to make sure microwave is operating at 30% power level-overheating will spoil the antigen and slides.
5. Remove from microwave and let cool in buffer at room temperature 10'
6. Rinse in gently-running tap water 10'
7. **2M HCl Brdu antigen retrieval 40'**
8. Wash with PBS 2 x
9. Lay slides out in hybridization box. Wash with 1x PBS x 2
   Use PAP pen to restrict subsequent volumes of reagents applied to the sections. Use water-soaked paper towels to create a moist atmosphere in the hybridization box.
10. Block with 1%BSA and 5% donkey serum 60' RT
11. Primary antibodies (1:250 in 0.5% BSA). Use 60 µL and put cover slips 4C overnight
12. Wash with 0.5% BSA x 3
13. Secondary antibodies cocktails solutions, e.g.:
    - donkey-anti-rabbit-488
    - donkey anti- rat-555
    - donkey anti-mouse-660
    - (1:1000) in 0.5% BSA
14. Incubate 1hr RT
15. Remove secondary Ab solution and add DAPI (200µL—incubate 15 min)
16. Wash with PBS x 5
17. Mount using Gelvatol mount solution