

Antibody staining on frozen sections

Tissue prep

- 1) Dissect tissue into cold PBS. For adult kidneys tissue should be cut.
- 2) Fix in 4% formaldehyde in PBS (prepared from a 37% stock solution) 1 hr, 4° with rocking.
(Notes: **4% PFA** also works fine for most antibodies. Fixation time likely varies by tissue and should be determined empirically. LacZ and GFP antibodies are fixation sensitive)
- 3) Wash 2 X 5 minutes in PBS 4° (several rinses of PBS is also fine)
- 4) Immerse in 15% sucrose in PBS, 4°, 4hrs or until tissues sinks (may be shorter)
- 5) Immerse in 30% sucrose in PBS, 4°, O/N
- 6) Embed in OCT, store blocks at -80
- 7) Cut 6-10um sections onto Superfrost/Plus slides (charged), store at -80.

Staining

- 1) Thaw slides ~10 minutes on benchtop, rinse 1 X 5min in PBS to remove OCT residue
- 2) Block tissue in 10% donkey serum in PBS OR **PBS-BB** (with or without DS), 60-90 minutes, RT. (PBS-BB→PBS+ 1%BSA, 0.2%Milk, 0.3% TritonX100)
- 3) Apply 1° antibody in 10% donkey serum in PBS OR PBS-BB, O/N, 4°
- 4) Wash 3 X 5 min PBS
- 5) Apply 2° antibody in PBS OR PBS-BB, 1hr, RT, in the dark
- 6) Wash 3 X 5 min PBS (If not using DAPI can stain with Hoestch nuclear dye, 1:500 in between washes)
- (6b) If needed, incubate with Streptavidin Reagent in PBS-BB, 30 min RT in the dark. Repeat washes
- 7) Mount with vectashield (+/- Dapi), coverslip and seal.

1° Antibodies

Rabbit $\alpha\beta$ Gal 1:5000(Cappel #55976, Lot 04993)
Mouse α Na/Katpase 1:250(could be further diluted, need to test) (Upstate #05-369)
Rat α PCAM 1:250 [endothelial cells] (BD biosciences #553370)
Hamster α Podoplanin 1:500 [glomerular epithelia] (Angiobio # 11033)
Rabbit α CLCKb 1:250 [thin limb]
Chicken α GFP (Aves) 1:500
Rabbit α GFP (Molecular Probes)1:200
Rabbit α Aqp1 1:1000

Rabbit α Aqp2 1:1000
Rabbit α Pax2 (Zymed) 1:200
LTL (FITC or Biotin conjugated) 1:200
Rabbit α THP (ascending limb) 1:100
Rabbit α WT1 (Santa Cruz) 1:100
Mouse α WT1 (Dako) 1:100
Goat α Jagged1 (Santa Cruz) 1:200
Rabbit α Cited1 (1:250)
Rat α Cyk8 (Troma-1 DSHB) 1:50

2° Antibodies @ (1:500) except for Cy5 Reagents @1:200

Protocol is also applicable to biotinylation. Use Vector Labs Biotin Block kit before blocking step.

Biotin conjugated 2° at 1:300
SA-488, 594 @ 1:1000; AMCA AvidinD (blue) @ 1:100.