IHC - Immunohistochemistry – from paraffin-embedded slides

1) Xylene washes (3 containers, #1, #2, #3, 3x5min) to de-paraffinize (xylene waste disposal in brown bottles under fume hood)
2) EtOH washes (100% x2, 95%, 70%, 50%) – 700ml 100% EtOH + 300ml H2O from big tank (in 50ml glass slide holders), 5x5min
3) PBS washes, 2x 5min
4) Antigen retrieval/unmasking
    a. Make buffer: 4.41g sodium citrate (our shelf) in 2l plastic container + 1l ddH2O from big tank, read ph (rinse first with ddH2O), bring PH to 6 by adding drops of 12N HCl using a transfer pipette, mix on shaker with a fish, add 500ml more ddH2O
    b. Pressure cooker: heat buffer for 2min (set time while blinking) at low pressure
    c. Put slides in black holder, close lid, put in buffer, heat for 5min at high pressure
    d. Release pressure, put pressure cooker bucket in sink with ice and let cool down for 15min.
5) Wash 2x 5min with PBS
6) 10% H2O2 (5ml hydrogen peroxide (Sheffield shelf) + 45ml PBS) – let slides sit for 20min – to inactivate endogenous peroxidase
7) Flick off H2O2, wipe slide on bottom, **circle** sample on top, add T-PBS (50microl Tween (improves permeability of cell membrane so that ab can enter the nucleus) in 50ml PBS = 0.1% T-PBS) – wash 3x 2min
   DON'T LET SLIDES DRY OUT
8) Add 5% Natural ….. Serum (against species of 2.ab) to block ??? (950microl PBS-T + 50microl serum (small brown bottle in fridge) – let slides sit for 45min.
9) Flick off and add avidin blocking solution – let sit for 15min.
10) 1x5min PBS

11) Flick off NGS, add a few drops biotin blocking solution – let it sit for 15min.
12) 2x5min PBS wash
13) Flick of and add 1.ab / NO AB for NEG CONTROL: mouse active b-catenin (in fridge, red lid), 1:300, (1000microl PBS + 3microl antibody) – incubate in fridge inside slide box overnight

---

14) Warm slides for 1h at 37C in small oven
15) 2x 5min PBS wash
16) 2.ab: biotinylated goat anti-mouse, 1:500, 2microl ab + 1000microl PBS-T – let sit 45min
17) 2x5min PBS wash
18) ABC kit: (avidin + biotinylated horseradish peroxidase H (HRP) – detects biotinylated 2.ab) – put a few drops of ABC reagent (grey bottle) on slides – let sit for 30min
19) 2x 5min PBS wash
20) DAB solution (peroxidase substrate to localize peroxidase) – in 15ml tube mix 5ml of ddH2O + 2 drops buffer + 4 drops DAB (diaminobenzidine – produces brown reaction product in presence of HRP) + 2 drops HP solution
21) Go over to microscope, turn on in the back, wheel on 0, yellow magnification
22) PC: camera control: live
23) Add DAB solution to slides and look under the microscope for brown stain
24) After 5-10min dump slides in glass jar with ddH2O to stop the reaction
25) Counter-stain with Hemotoxylin – transfer slides for 30sec into glass jar with H (from under the hood) and then back into jar with ddH2O, recycle H back into big glass jar, wash slides inside the glass jar with tab water to bring out blue stain (nucleus), add a little bit of ddH2O from glass jar to slides and look under microscope
26) EtOH dilutions for de-hydration: add 50% EtOH into glass jar, let sit 5x5min., then 70%, 90%, 100%

27)Xylene 1x10min in jar#3

28)Mounting