

DAB BrdU ICC

	Steps	Notes
	Fix fresh frozen sections:	
<input type="checkbox"/>	Dry sections for 30 mins after cutting or for 15mins from -80 freezer.	
<input type="checkbox"/>	Fix sections in 4% PFA for 10 mins	
<input type="checkbox"/>	Wash 3 times, 5 mins each in PBS, rocking platform.	
	Post-fix protocol- Day 1:	
<input type="checkbox"/>	Before anything else, start preheating 2M HCl (45-60 minutes)	
<input type="checkbox"/>	Place slides in PBS for 5 minutes	
<input type="checkbox"/>	PBS wash (5min)	
<input type="checkbox"/>	Shake off PBS before acid step so acid isn't diluted. Incubate in 2M HCl at 37C for 30 mins (with preheated HCl!)	
<input type="checkbox"/>	Put slides into 0.1M Sodium borate for 10 mins at room temp.	
<input type="checkbox"/>	PBS wash (5min with shaking)	
<input type="checkbox"/>	0.2% H2O2 in PB (NOT PBS!) 20 minutes	
<input type="checkbox"/>	PBS wash (5min)	
<input type="checkbox"/>	Place in Rack with PBS	
<input type="checkbox"/>	PBS wash (5min)	
<input type="checkbox"/>	Add blocking solution (0.3% triton and 5% serum in PBS) to sections. Use 400 μ l per slide. **Animal serum used should correspond to the animal the 2 ^o Ab is made in**	
<input type="checkbox"/>	Leave at room temp for 30 mins in rack	
<input type="checkbox"/>	Add primary Ab: Rat anti-BrdU Ab at 1:200 in blocking solution. Use 400 μ l per slide.	
<input type="checkbox"/>	Place in the cold room overnight.	
	Day 2:	
<input type="checkbox"/>	Dump out liquid from the bottom of the rack	
<input type="checkbox"/>	Wash 3 times with PBS (10 mins)	
<input type="checkbox"/>	Add secondary Ab: Goat anti-rat at 1:200 in 0.3% triton in PBS (no serum). Use 400 μ l per slide to cover all sections.	
<input type="checkbox"/>	Incubate 2 hours at room temp.	
<input type="checkbox"/>	Dump out liquid from the bottom of the rack	
<input type="checkbox"/>	Wash 3 times with PBS (5 mins)	
<input type="checkbox"/>	ABC (500uls -30 min) (kept in walkin cooler)	
<input type="checkbox"/>	Wash 3 times with PBS (5 mins)	
<input type="checkbox"/>	Set up for DAB step in the hood (Take out DAB from -80 and thaw. 58 ml of PB and 2mls of DAB and 20ul of 30% H2O2 VERY last!!). Mix DAB and PB and then filter using a 60ml syringe with a blue 0.2ul filter into marked DAB slide container	
<input type="checkbox"/>	Slip slides into the container and THEN add the H2O2	
<input type="checkbox"/>	H2O2 step for 7 minutes	
<input type="checkbox"/>	PBS wash (1min) to rinse	
<input type="checkbox"/>	PBS wash (2min)	
<input type="checkbox"/>	70% ethanol (1min: CLEAN)	
<input type="checkbox"/>	95% ethanol (1min: CLEAN)	
<input type="checkbox"/>	100% ethanol (1min: CLEAN)	
<input type="checkbox"/>	Xylene (5min)	
<input type="checkbox"/>	Coverslip with krystalon	
<input type="checkbox"/>	Clean all DAB containers with diluted bleach and rinse with water really well!	

Solutions	Notes
4% PFA in PBS (500ml)	
Heat 200ml dH ₂ O to about 56 deg in a beaker with stir bar	
Add 20g PFA powder	
Once mixed, add 2 drops 10M NaOH	
Do not let temp reach 60 deg	
Once clear, add 250ml PB (200ml dibasic + 50ml monobasic)	
Check neutral pH with litmus	
Filter solution	
2M HCl (make fresh)	
84ml dH ₂ O	
16ml conc HCl	
Add HCl to H ₂ O in fume hood.	
0.1M Sodium Borate (1 L)	
Dissolve 38.137 g Na borate in 500ml dH ₂ O with fast stirring	
Once dissolved add conc HCl to pH 8.5 (about 10ml)	
Add more dH ₂ O to male 1 L	
Blocking solution (make 800μl/slide)	
0.3% Triton X into PBS in a 15ml falcon tube (i.e. 30 μ l in 10ml). Cut end of pipette tip to add triton.	
Vortex and leave to shake for 30 mins	
Immediately before blocking, add 5% serum and keep on ice. Thaw serum on ice beforehand. Can keep extra serum in fridge for maximum 1 week.	
0.2% H2O2	
67ul of 30% H2O2	
100ml of PBS	