6/7/2010 KW

IHC protocol for mPygo2

A. Deparaffiniazation~rehydration

1. Xylene: 2 x 10 min
2. 100% EtOH: 2 x 3 min→95% EtOH: 5 min→70% EtOH: 5 min→ 50% EtOH: 5 min→Running water 5 min

B. Antigen retrieval

1. Microwave in Tris-EDTA buffer (10 mM Tris, 1 mM EDTA, 0.05% Tween20, pH9.0): 20 min (start with 100 mL and add ~50 mL every 5 min to avoid drying up)
2. Running water 5 min

C. Immunostaining

1. Wash in TBS-t: 2 x 5 min
2. Mark around the tissue with pap-pen
3. Block in 10% normal goat serum and 1% BSA in TBS: 2 h at RT
4. primary antibody (rabbit anti-Pygo2 homemade antibody, 1:2000 in TBS with 1% BSA): overnight at 4 ºC
5. Wash in TBS-t: 2 x 5 min
6. Incubate in 0.3% H2O2 in TBS: 15 min at RT
7. Wash in TBS-t: 2 x 5 min
8. secondary antibody (biotinylated anti-rabbit IgG (Vector), 1:200 in TBS with 1% BSA): 45 min at RT
9. Wash in TBS-t: 2 x 5 min
10. Vector ABC kit (20 μL A sol. + 20 μL B sol. in 1 mL DDW, prepare at least 30 min before use) : 30 min at RT
11. Wash in TBS-t: 2 x 5 min
12. DAB staining (Dako, 20 uL DSB in 1 mL Substrate buffer) : ~5-10 min at RT
13. Running water 10 min

D. Counter staining~ dehydration~ mounting

1. Hematoxylin : 1 min at RT
2. Running water 10 min
3. 95% EtOH: 1 min→100% EtOH: 2 x 5 min
4. Xylene : 2 x 5 min at RT
5. Mount in Permount