

Sclerostin IHC protocol from Paola Divieti-Pajevic

1. Incubate slides at 60C for an hour on a slide warmer
2. Deparaffinize and hydrate (xylene 3x 5', EtOH 3x 2')
3. Incubate 1x TBS 5'
4. Antigen retrieval with proteinase K (20 ug/ml) for 15' at RT in water
5. Quench in 3% H₂O₂/MeOH for 10' (25 ml 30% H₂O₂, 225 ml MeOH)
6. Wash dH₂O 5'
7. Circle section with PAP-pen, rinse 1x TBS
8. Block slides in 100 ul TNB 30' in humidified chamber
9. Remove blocking buffer, add primary antibody (biotinylated anti-sclerostin 1:50, R+D BAF1589) diluted in TNB for 1hr
10. Wash in TBST 3x 5'
11. Add 100 ul SA-HRP diluted 1/100 in TNB for 30'
12. Wash TBST 3x 5'
13. Add 100 ul biotinyl tyramide (diluted 1/50 in amplification diluent) for 5'
14. Wash TBST 3x 5'
15. Add 100 ul SA-HRP diluted 1/100 in TNB for 30'
16. Wash TBST 3x 5'
17. For HRP detection add 150 ul DAB
For Vector DAB: 5ml H₂O, 2 drops buffer, 4 drops DAB, 2 drops H₂O₂, 2 drops NiCl (optional)
18. Incubate 2-10' until color develops
19. Wash 5' H₂O
20. Counter stain with methyl green, coverslip with Histomount