VEGFA IHC Protocol

1. Put slides in 37°C overnight, or 60°C for an hour
2. Deparaffinize and hydrate (xylene 3x 5’, EtOH 3 x 2’)
3. Quench in 0.3% H₂O₂/MeOH for 20’ (on shaker)
4. Wash with PBS 5’ (on shaker)
5. Antigen retrieval with proteinase K 10 mcg/ml in 10 mM Tris pH 7.6 for 25 mins RT
6. Wash 5’x3 with PBS(on shaker)
7. Circle with PAP pen, rinse with TBS
8. Block slides with ~100 ul TNB in a humidified chamber at RT for 30’
9. Incubate slides with TNB + anti VEGFA 1:1000 (Abcam ab46154) in TNB in humidified chamber at 4C overnight
10. Wash in TBST 3x 5’ (on shaker)
11. Incubate in humidified chamber with biotinylated anti-Rabbit (1:2000) in TNB for 60’ at RT
12. Wash in TBST 3x 5’ (on shaker)
13. Incubate with SA-HRP (1:100) in TNB, in humidified chamber for 30’ at RT
   a. SA-HRP is from the Perkin Elmer Kit: TSA Biotin System NEL700A001KT
14. Wash in TBST 3x 5’ (on shaker)
15. Add 100ul biotinyl tyramide (diluted 1:50 in amplification diluents) for 5’ in humidified chamber at RT
   a. Biotinyl tyramide and amplification diluents are from the Perkin Elmer Kit
16. Wash in TBST 3x 5’ (on shaker)
17. Incubate with SA-HRP (1:100) in TNB, in humidified chamber 30’
18. Wash 3x TBST (on shaker)
19. DAB detection (Peroxidase Substrate Kit: DAB SK-4100, Vector Laboratories Inc.)
   a. To 5 ml H₂O add the following from the DAB kit: 2 drops buffer, 4 drops DAB, 2 drops H₂O₂)
   b. Add ~100 drop wise to each sample ~90”, wait until a clear change in color is seen (will turn brown), Once color change occurs place slides in water
20. Wash 3’ H₂O (on shaker)
21. Counterstain and coverslip

May only need one amplification.

TNB: 50 mg blocking reagent (from Perkin Elmer Kit) + 10 ml 1x TBS, vortex and heat at 37°C to dissolve
10x TBS: 250 ml 1M Tris, 150ml 5M NaCl, 100 ml H₂O
10x TBST: 200 ul 8M Tris, 400 ml 5M NaCl (pH8), 5 ml Tween20, 595 ml H₂O
- Perkin Elmer Kit: TSA Biotin System NEL700A001KT
- Peroxidase Substrate Kit: DAB SK-4100, Vector Laboratories Inc.