

## Center for Musculoskeletal Research

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## Histology and Histomorphometry Core

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### VEGFA IHC Protocol

1. Put slides in 37C overnight, or 60C for an hour
2. Deparaffinize and hydrate (xylene 3x 5', EtOH 3 x 2')
3. Quench in 0.3% H<sub>2</sub>O<sub>2</sub>/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 H<sub>2</sub>O add the following from the DAB kit: 2 drops buffer, 4 drops DAB, 2 drops H<sub>2</sub>O<sub>2</sub>
  - 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' H<sub>2</sub>O (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 37C to dissolve

**10x TBS:** 250 ml 1M Tris, 150ml 5M NaCl, 100 ml H<sub>2</sub>O

**10x TBST:** 200 ul 8M Tris, 400 ml 5M NaCl (pH8), 5 ml Tween20, 595 ml H<sub>2</sub>O

- Perkin Elmer Kit: TSA Biotin System NEL700A001KT

- Peroxidase Substrate Kit: DAB SK-4100, Vector Laboratories Inc.