

## Center for Musculoskeletal Research

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## Histology and Histomorphometry Core

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### Immunohistochemistry: CD31 (by Masanobu Ohishi)

#### Tissue Preparation:

1. Fix the bone samples in 4% PFA for 24 hrs at 4°C
2. Decalcify the samples with 20% EDTA at 4°C for 7 days (change the EDTA daily)
3. Paraffin processing (histocore)

#### Before starting:

- Make 1l 1X TBS
  - o 100 ml 10X TBS
    - 10X TBS (1l): 60.5 g Tris (Fisher BP152)  
87.6g NaCl (Fisher BP358)  
pH to 7.5  
Bring to 1l w/ ~800 ml H<sub>2</sub>O
  - o 900 ml H<sub>2</sub>O
- Prepare humid chambers (w/ Whatman paper)
- Make 4l 1X TNT
  - o 400 ml 10x TBS
  - o 3600 ml dH<sub>2</sub>O
  - o 2 ml Tween-20 (Sigma P2287): add while stirring
- Make Proteinase K
  - o 10mg/ml in 10mM Tris pH 7.5/1mM CaCl<sub>2</sub>

#### Staining process: (use TSA kit, Perkin Elmer #NEI700A)

1. Incubate slides at 60°C on slide warmer for at least 1 hr
2. Deparaffinize and dehydrate (use clean solutions!)
  - a. Xylene, 3x 3 min
  - b. 100% EtOH, 2x 2min
  - c. 95% EtOH 2 min
3. Rehydrate in 1X TBS, 5 min RT
4. Circle sections with Pap=pen
5. Proteinase K (Sigma P2308) 20  $\mu$ g/ml, 15 min RT in moist chamber
  - a. 6  $\mu$ l of 10mg/ml in 3 ml dH<sub>2</sub>O

2 mg/ml stock sol'n  
2000  $\mu$ g/ml  
↓ 100x  
20  $\mu$ g/ml

6. 3% H<sub>2</sub>O<sub>2</sub>/MeOH 10 min RT
  - a. 25 ml 30% H<sub>2</sub>O<sub>2</sub>+ 225 ml MeOH
7. dH<sub>2</sub>O, 5 min, RT
8. Equilibrate sections by rinsing with 1X TBS
9. Incubate with TNB at RT in moist chamber for 30 min
10. 1° antibody diluted in TNB, RT in moist chamber for O/N at 4°C
  - a. CD31 (BD 550274) 1:200 dilution in TNB
11. Wash: 1X TNT, 3x 5 mins at RT

12. Biotinylated 2° Ab diluted in TNB, in moist chamber for 30 min
  - a. antiRat IgG, 1/300 (Dako E0468)
13. Wash: 1X TNT, 3x 5 min at RT
14. SA-HRP (from kit) 1:100 dilution in TNB, 30 mins at RT
15. Wash: 1X TNT, 3x 5 min at RT
16. Biotynil Tyramid (kit) 1:50 dilution in amplification diluent, 5 min RT
17. Wash: 1X TNT, 3x 5 min at RT
18. SA-HRP (from kit) 1:100 dilution in TNB, 30 min RT
19. Wash: 1X TNT, 3x 5 min at RT
20. For HRP detection, use DAB (VECTOR SK-4100)
  - In 5ml dH<sub>2</sub>O:
    - Add 2 drops buffer; mix well
    - Add 4 drops DAB; mix well
    - Add 2 drops H<sub>2</sub>O<sub>2</sub>; mix well
    - Add 2 drops NiCl (optional)
21. Incubate until color develops (2-10 mins)
22. Wash 5 mins in dH<sub>2</sub>O
23. Counterstain (optional)
24. 95% EtOH 2 min
25. 100% EtOH 2x 3 min
26. Xylene 2x 5 min