

Center for Musculoskeletal Research

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Histology and Histomorphometry Core

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IHC protocol anti-MMP13 (rabbit polyclonal ab39012-abcam) (Eva Liu)

Day 1

Paraffin Sections

1. Dry slides Over Night in 37C Hand heat paraffin for 1hr at 55C to melt wax
2. Deparaffinize slides in 2 changes of xylene x 5min each
3. Rehydrate in graded alcohol 100% → 95% → 70%, 3min each
4. PBS wash 5min
5. Antigen retrieval:
10 min: sodium citrate buffer 95-97C (water bath=100), then cool 30 min
6. Wash PBS
5. Incubate slides in 3% H2O2 in methanol: 20min
6. PBS Wash 5 min
8. Block slides 10% HIFBS/PBS 60 min before primary antibody
9. Primary AB 1:200 in 10%HIFBS/PBS 4C overnight

Day 2

10. TBST wash 3x 5min each
11. Secondary AB (1:400 anti-rabbit HRP) in 10%HIFBS/PBS 1HR RT
12. TBST wash 3x 5min
13. DAB Detection
 1. 5ml ddH2O
 2. 2 drops buffer → VORTEX
 3. 4 drops DAB → VORTEX
 4. 2 drops H2O2 → VORTEX
20. Stop reaction in ddH2O
21. Counterstain with Fastgreen 1 min → Acetic Acid 30 sec → ddH2O
22. Dehydrate in graded alcohol 70% → 95% → 100% → Xylene
23. Coverslip