

Center for Musculoskeletal Research

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Histology and Histomorphometry Core

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IHC protocol NOGGIN amplified (goat polyclonal anti-mouse Noggin AF719 R&D)

Day1

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. Trypsin 6 min -7 min @37C
6. Wash PBS
5. Incubate slides in 3% H2O2 in methanol: 20min
6. PBS Wash 5 min
8. Block slides in 100mcl of TNB 30 min
9. Primary AB 1:100 in TNB 4C overnight

Day 2

10. TBST wash 3x 5min each
11. Secondary AB (1:300 anti-goat HRP OR 1:300 biotinylated anto goat AB) in TNB 1HR RT
12. TBST wash 3x 5min each and go to DAB (#15)
13. In Biotinylated AB use after wash : SA-HRP (diluted 1:100 in TNB) 30min RT
14. TBST wash2x 5min each
15. 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