

Leslie Slota

Cohn Laboratory

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## **Alcian Blue Staining for horseshoe crab paraffin-embedded sections**

-to stain sulfated glycosaminoglycans in horseshoe crab embryos and adult tissues

1. Deparaffinize sections to ddH<sub>2</sub>O
  - a. Xylene 2 times 5 min each
  - b. 100% Ethanol 10 min
  - c. 95% Ethanol 10 min
  - d. 70% Ethanol 10 min
  - e. deionized water 5 min
2. 1% Alcian Blue 30min
3. Deionized water 2 min
4. Sigma-Aldrich Nuclear Fast Red 5 min
5. Deionized water 1 min
6. Dehydrate in alcohols
  - a. 100% ethanol 2 times 1 min each
7. Clear with 2 changes xylene 1 min each
8. Mount with paramount mounting medium

1% Alcian Blue Solution:

Add 1 gram of 8GX Alcian blue powder to 100 mL of a 3% acetic acid solution.