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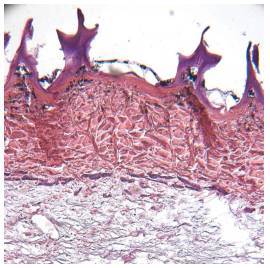
Hematoxylin & Eosin Histology of Elasmobranch skin



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We use this protocol extensively in adult elasmobranch skin tissues.

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Abstract

Hematoxylin and eosin stain is probably the most used staining method in histology. It provides a comprehensive and detailed picture of different types of cells and tissues. Preparing hard dermal tissues for H&E requires special steps. In this protocol, we describe what we found to work best for staining Elasmobranch skin and aim.

Image Attribution

Rebecca Lin

Materials

- Preserved hatchling and adult Squalus from Carolina Biological Supplies
- Preserved Leucoraja erinacea hatchling from Carolina Biological Supplies, Woods Hole Marine Laboratories
- Live Urobatis halleri from Santa Monica Bay
- 4% Paraformaldehyde (PFA)
- 50mL Conical tubes
- Tricaine (MS-222)
- 5M ethylenediamine tetraacetic acid
- 50mL Conical Tube
- Methanol (Alfa Aesar, Lot #10208556)
- 100% Ethanol (PHARMCO 200 proof, catalog #111000200)
- Histosol (National Diagnostics, catalog #50-899-901
- Paraffin (Leica Paraplast Plus, catalog #19216)
- 50mL glass beaker (Pyrex, Product #1000)
- Weight boat
- Vacuum oven (National Appliance Co., Model #5830)
- Degassed ultra-pure water
- Microscope slides (Fisherbrand, Superfrost Plus, catalog #22-037-246)
- Microtome (Spencer, Model #820)
- Transfer pipette.
- Slide warmer (Fisher Scientific, Model #26020FS)
- Small paint brushes
- Tweezers
- Glass staining dish with removable rack
- 100% Ethanol (PHARMCO 200 proof, catalog #111000200)
- 95% Ethanol (PHARMCO 190 proof, catalog #111000190)
- Xylene (Fisher Scientific, Lot #114268)
- De-ionized water
- Hematoxylin solution (StatLab, Lot #185792)
- Eosin Y solution (StatLab, Lot #188123)
- Vintage Bluing (StatLab, Lot #187670)
- Paraffin Slides
- Cover glass (Fisherbrand, Lot #21824)
- Transfer pipette
- Permount mounting medium (Fisherbrand, Lot #014923)

Fixation:

1 Squalus and Leucoraja were initially preserved in formalin by Carolina Biological Supplier. We post-fixed tissues in 4% PFA for a minimum of 24 hours to improve the fixation of skin structures. We dissected 2cm blocks of skin from the head and trunks and kept them in 4%PFA. Live *Urobatis halleri* and *Leucoraja* hatchlings were anesthetized and euthanized with 1g/L tricaine (MS-222) until 10 minutes past cessation of gilling, and 2cm blocks of head and trunk skin fragments were dissected and immediately preserved tissues in 4% PFA for storage.

Demineralization:

2 Cut the shark tissue into a 2 x 2 cm cube shape, then place it in a 50 mL conical tube. Add 5M ethylenediamine tetraacetic acid (EDTA) solution to submerge the tissue sample for 3 days.

Tissue Embedding in Paraffin:

3 Decant the EDTA and replace it with ice-cold methanol to submerge the sample. Then, place the Conical tube in a freezer at -20°C for at least 12 hours. Based on our prior experiences, leaving the sections for more extended periods is acceptable without causing a problem to the tissue.

4 Remove the Conical tube from the freezer, decant the methanol, and add 100% ethanol to submerge the section. Keep the sample on a shaker at a moderate speed. Change the ethanol at least twice a day for 3-5 days. The aim is to have the tissue at 0% H_2O and 100% dehydrated. This is crucial for the best paraffinization.

5 After 3-5 days of exposure to ethanol,remove the ethanol and replace it with histosol. Change the histosol 4 times or more to ensure that ethanol is removed. Leave the sample in histosol until the section becomes partially transparent. *This step should be done in a safety hood.*

6 Once the sample is ready, transfer the sample with histosol to a 50mL beaker and place it in a vacuum oven (59-62°C) to warm it up. Remove half of the histosol and replace it with an equal volume of melted warm paraffin. Place the beaker in the vacuum oven.

7 We repeated step 1, removing 2/3 of the paraffin and histosol mixture twice a day and replacing it with melted paraffin until no smell of histosol remained. Total removal of histosol may take up to 3-5 days, depending on sample size. It is important not to rush this process to ensure the quality of the sample.

8 Once there is no longer any scent of histosol, the sample is ready to be put in a paraffin mold. To embed the sample, pour a thin layer of melted paraffin into a weight boat and wait for 5-10 seconds until the paraffin starts to solidify. Use a tweezer to gently transfer the sample into the

weight boat. Add more melted paraffin to submerge the sample. Ensure the sample does not touch the weight boat's bottom or edges. The sample is ready for microtome once it is fully solidified.

Sectioning Tissue Using a Microtome

9 Set the slide warmer to 37.5°C. Place some Superfrost slides on the slide warmer and use a transfer pipette to gently transfer enough degassed ultra-pure on the slide so the sectioned tissue can float on it. Fisherbrand Superfrost plus microscope slides are strongly recommended for the best results. Other brands may pose issues, such as tissues falling off the slide during the staining process.

10 Set up the microtome for sectioning. Insert the blade, adjust the angle, and set the desired section thickness. The blade should cut straight across the block. Once everything is set, insert the paraffin block and carefully trim it until the tissue surface is exposed.

11 Start sectioning, then use two small paintbrushes or a tweezer to transfer the section on the slide. Each slide should have 6-8 sections depending on the size, and sections should be floating on the water. Leave the slides on the slide warmer to dry, then label the slides with appropriate information.

Hematoxylin and Eosin Staining

12 *We use staining dishes with a stain dipper. All the solutions are in staining dishes, and slides are placed in the stain dipper. We transferred the stain dipper from each staining dish in the following sequence and time:*

12.1 Deparaffinization:
Place the rack in the first xylene dish for 2.5 minutes,transfer to the second xylene dish for 2.5 minutes, then transfer to the third xylene dish for 2.5 minutes.

12.2 Rehydration:
Transfer the rack to the first 100% Ethanol dish for 2.5 minutes, then 2.5 minutes in the second 100% Ethanol dish, followed by transferring the slides to the third 100% Ethanol dish for an additional 2.5 minutes. Then, the slides go through 95% Ethanol twice, for 2.5 minutes each. Place the rack in fresh DI water for 2 minutes.

12.3 Hematoxylin Staining (nuclear stain):
Dry excess water before placing the rack in Hematoxylin.
Place the rack in Hematoxylin for 1 minute, then rinse in fresh DI water for 2 minutes. After rinsing in water, place the rack in vintage bluing for 4 minutes, then rinse in fresh DI water for 2 minutes. This step converts the Hematoxylin to a dark blue color.

12.4 Eosin Counterstaining (non-nuclearelement):
Place the rack in 95% Ethanol for 2 minutes, then in Eosin solution for 5 seconds.

12.5 Dehydration and Mount:
The slides go through 95% Ethanol three times, 1 minute each, followed by 3 times 100% Ethanol, 2
minutes each, followed by 3 times xylene, 3 minutes each. Drain the slides, then use a transfer pipette to place 2-3 drops of Permount onto each slide. Gently place a coverslip on top, taking care to avoid bubbles. Allow the slides to dry overnight in the safety hood.

Protocol references

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