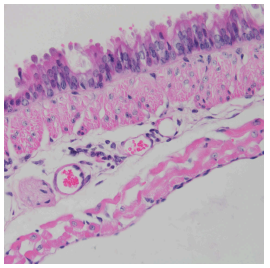
Mar 20, 2025

Haematoxylin and Eosin (H&E) staining of paraffin sections



DOI
https://dx.doi.org/10.17504/protocols.io.n92ld5b3xv5b/v1

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OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.n92ld5b3xv5b/v1

Protocol Citation: Samson Dowland 2025. Haematoxylin and Eosin (H&E) staining of paraffin sections. **protocols.io** https://dx.doi.org/10.17504/protocols.io.n92ld5b3xv5b/v1

Protocol status: Working
We use this protocol and it's working

Created: March 16, 2025

Last Modified: March 20, 2025

Protocol Integer ID: 124459

Keywords: Histology, Staining, Microscopy, Pathology, Haematoxylin, Eosin, initial red colour of the haematoxylin, haematoxylin dye, rich contrast to the other tissue component, histological staining technique, used histological staining technique, paraffin sections haematoxylin, staining biological sample, stained cytoplasm, alum haematoxylin, rich in proteoglycan, erythrocyte, nuclear staining, other tissue component, haematoxylin, clear overview of cellular structure, proteoglycan, tissue for diagnostic purpose, cytoplasm of most other cell type, other cell type, differentiation between cytoplasm, most other cell type, tissue architecture, routine histopathological examination, cellular structure, cell, cytoplasm, standard choice for routine histopathological examination, biological samples for biomedical research, eosin, connective tissue, tissue, bluing solution, areas of bone, cartilage, initial red colour, staining of paraffin sections haematoxylin, staining, alkaline ph of scott, cell type

Abstract

Haematoxylin and Eosin (H&E) staining is the most-commonly used histological staining technique. This method provides a clear overview of cellular structure and tissue architecture, making it the standard choice for routine histopathological examination of tissue for diagnostic purposes and a popular option when staining biological samples for biomedical research or teaching purposes.

The Harris's Haematoxylin used here is an alum haematoxylin, meaning it uses an aluminium salt as a mordant. Once nuclear staining is complete, the alkaline pH of Scott's bluing solution is used to convert the initial red colour of the haematoxylin to a light blue. Areas of bone and cartilage that are rich in proteoglycans will also take up the haematoxylin dye and stain blue-purple. Subsequent counterstaining with the combined Eosin Y + Phloxine B solution provides rich contrast to the other tissue components, enhancing differentiation between cytoplasm, muscle and connective tissue. In particular, erythrocytes are stained vibrantly and appear almost red, making them stand out against the pink-stained cytoplasm of most other cell types.

Protocol materials

- Hydrochloric Acid **Banksia Scientific Company (AJAX FineChem) Catalog #**AJA256
- Ethanol **POCD Scientific Catalog #**ETHABS70WV5
- Eosin Alcoholic 0.1% with Phloxine (SNP Formula) **POCD Scientific Catalog #**SNPEOSIN5L
- Ethanol **Hurst Scientific Catalog #**EIMS95-5L
- Ethanol **POCD Scientific Catalog #**ETHABS20M
- Xylene **POCD Scientific Catalog #**XYL5P
- DPX Mountant for histology **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**06522
- Harris Haematoxylin **POCD Scientific Catalog #**HHXIMP
- Sodium hydrogen carbonate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**401676
- Magnesium sulphate **ChemSupplyAustralia Catalog #**ML073

Deparaffinisation and rehydration

32m

1 **Dewax and rehydrate tissue sections** 32m

Nuclear Staining

5m 30s

2 Stain in Harris's Haematoxylin 2m

Harris Haematoxylin **POCD Scientific Catalog #**HHXIMP

3 Wash with running tap water to remove excess stain 2m

4 Differentiate in acid alcohol (5 - 10 dips)

[1% Hydrochloric Acid (HCl) in 70% Ethanol]

Hydrochloric Acid **Banksia Scientific Company (AJAX FineChem) Catalog #**AJA256

Ethanol **POCD Scientific Catalog #**ETHABS70WV5

5 Wash in tap water 30s

6 Blue in Scott's Bluing Solution 30s

[Sodium hydrogen carbonate 2.5g/L; Magnesium sulphate 20g/L; in distilled water]

Sodium hydrogen carbonate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**401676

Magnesium sulphate **ChemSupplyAustralia Catalog #**ML073

7 Wash in tap water 30s

8 Examine slides under microscope to assess nuclear staining. Nuclei should be blue, cytoplasm and collagen should be grey. If sections remain overstained (cytoplasm and other tissue components remain blue/purple) repeat steps 4-8. If sections have been over-differentiated, return to step 2.

Counterstaining

1m 20s

9 70% Ethanol

Ethanol **POCD Scientific Catalog** #ETHABS70WV5

10 Counterstain in Eosin - 2 changes, 40 seconds each

1m 20s

Eosin Alcoholic 0.1% with Phloxine (SNP Formula) **POCD Scientific Catalog** #SNPEOSIN5L

Dehydration, clearing and mounting

16m

11 95% Ethanol

1m

Ethanol **Hurst Scientific Catalog** #EIMS95-5L

12 95% Ethanol

1m

Ethanol **Hurst Scientific Catalog** #EIMS95-5L

13 100% Ethanol

2m

Ethanol **POCD Scientific Catalog** #ETHABS20M

Note: ensure whole slide and slide rack is fully immersed in ethanol to remove all traces of water, preventing water contamination of the xylene

14 100% Ethanol

2m

Ethanol **POCD Scientific Catalog** #ETHABS20M

Note: ensure whole slide and slide rack is fully immersed in ethanol to remove all traces of water, preventing water contamination of the xylene

15 100% Xylene 5m

Xylene **POCD Scientific Catalog #**XYL5P

16 100% Xylene 5m

Xylene **POCD Scientific Catalog #**XYL5P

17 Coverslip with a resinous mounting medium, such as DPX

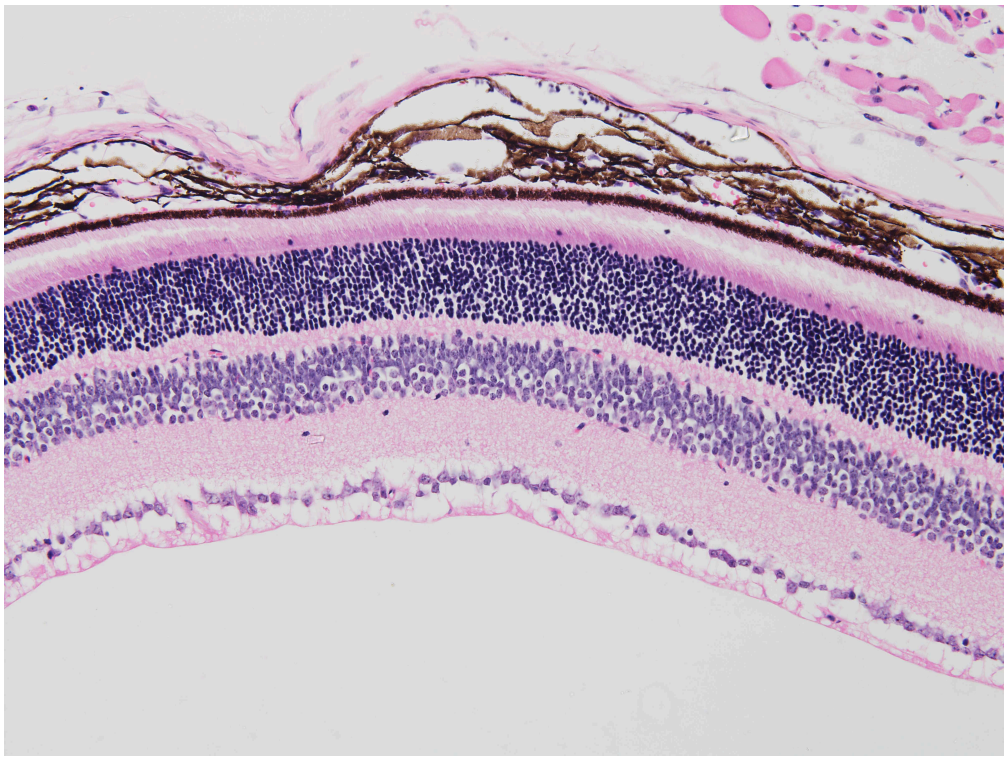
DPX Mountant for histology **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**06522

Results 54m 50s

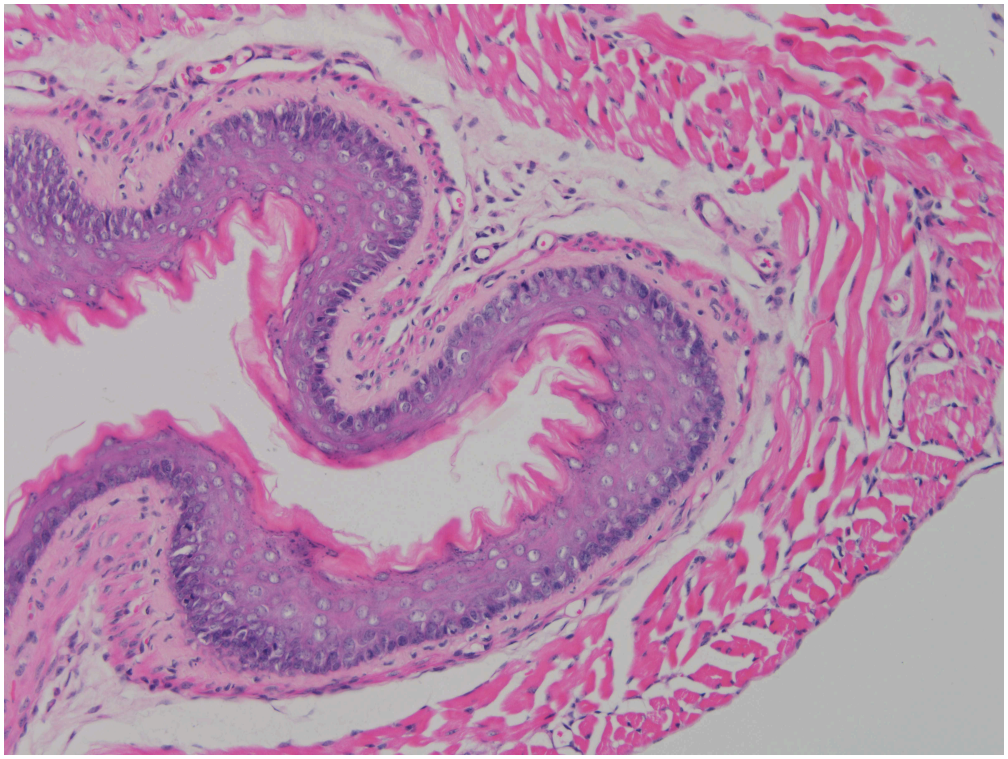
18

Expected result

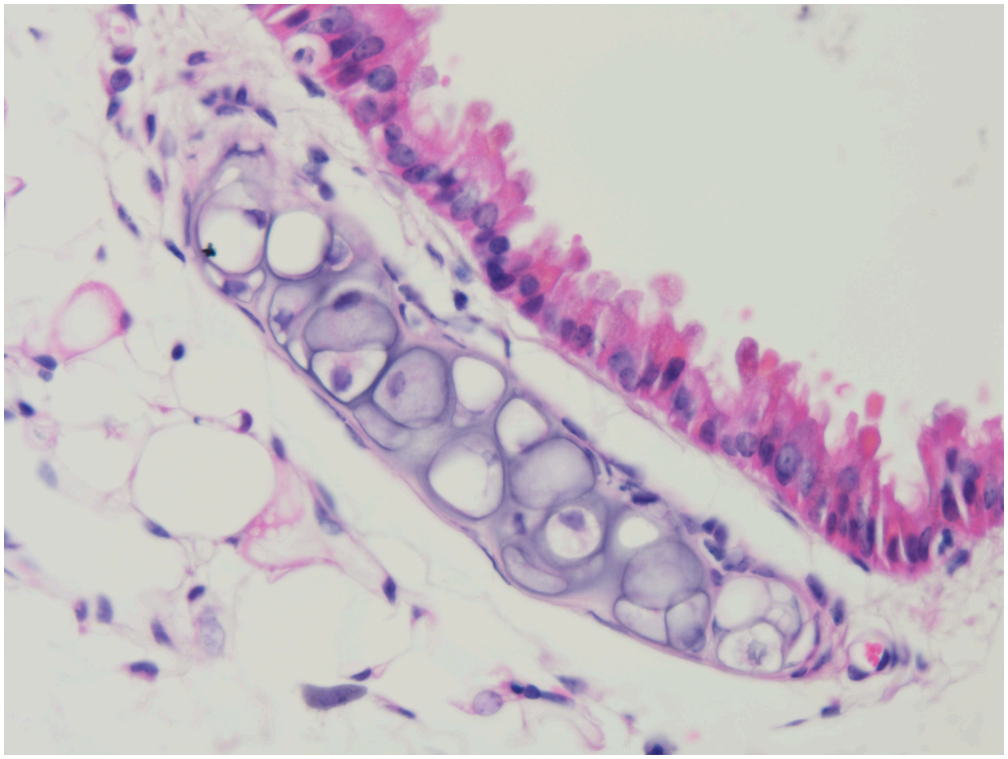
Nuclei: blue-purple
Cytoplasm: pink
Erythrocytes: pink-red
Collagen: pink
Muscle: pink-red
Cartilage: pink-blue
Bone: pink-purple



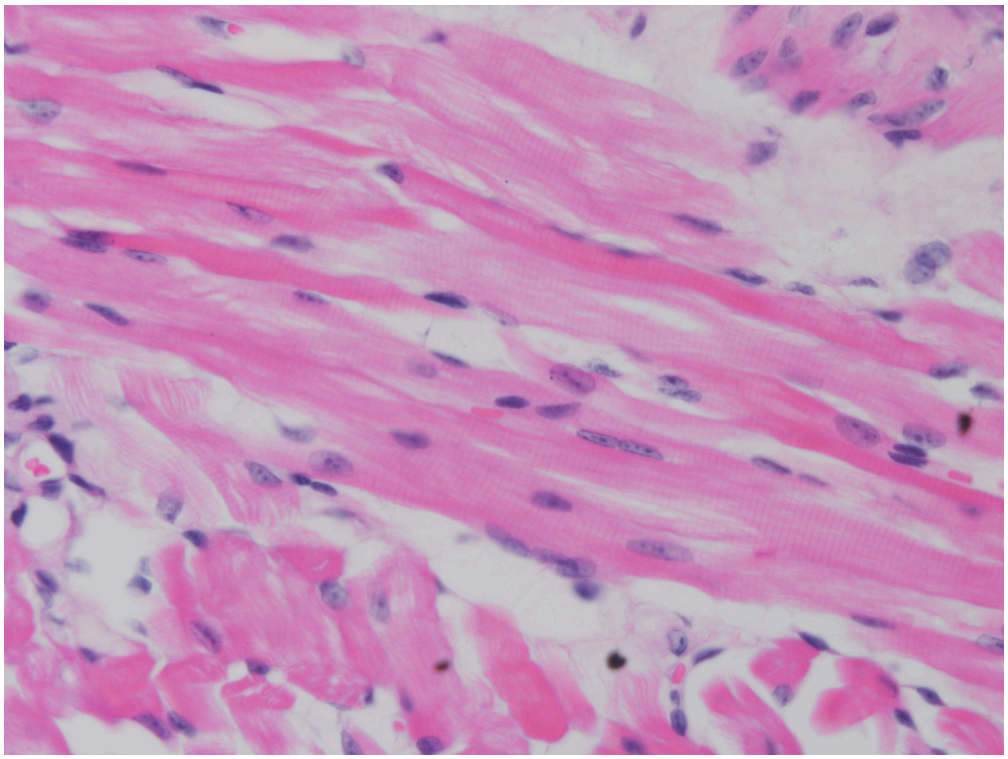
Mouse retina



Mouse oesophagus. Demonstrates staining in keratin, epithelium, connective tissue and muscle.



Mouse bronchus, demonstrating staining of cartilage.



Mouse skeletal muscle.

Protocol references

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Avwioro, G. (2011). Histochemical uses of haematoxylin—a review. *Jpcs*, *1*(5), 24-34.
https://www.stainsfile.com/theory/methods/hematoxylin-eosin-staining/eosin/