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Verhoeff's Iron Hematoxylin Staining

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1 Works for me dx.doi.org/10.17504/protocols.io.be9sjh6e

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ABSTRACT

This protocol is used to stain elastic tissue.

This results in elastic fibers and nuclei stained black.

Staining of elasin allows for quantification of elastin breaks.

EXTERNAL LINK

<http://cvrc.med.uky.edu/lab-protocols>

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Chen JZ, Sawada H, Moorlegghen JJ, Weiland M, Daugherty A, Sheppard MB. Aortic Strain Correlates with Elastin Fragmentation in Fibrillin-1 Hypomorphic Mice. Circ Rep. 2019;1(5):199–205.
doi:10.1253/circrep.CR-18-0012

ATTACHMENTS

[Verhoeff's Iron Hematoxylin Staining.pdf](#)

MATERIALS TEXT

1. Verhoeff's iodine (50 ml stock solution)

Iodine - 1 g

Potassium iodide - 2 g

Distilled water - 50 ml

2. Verhoeff's iron hematoxylin (36 ml)

Hematoxylin (5% wt/vol) in absolute alcohol - 20 ml

Ferric chloride (10% wt/vol) - 8 ml

Verhoeff's iodine - 8 ml

Mix a fresh solution, in the order given for each usage.

3. Ferric chloride (2% wt/vol)

4. Alcohol (95% vol/vol)

Acetone Fischer S25120A

Iodine Fischer I35-100

Potassium Iodide Sigma P8256

Hematoxylin Sigma H9627

Ferric (III) Chloride Sigma F2877

100% ethanol Fischer BP2818-4

Xylene Millipore XX0060-4

BEFORE STARTING

Place slides in glass holder. Be sure that slides are labeled appropriately.

Prepare reagents before starting.

Elastin Staining

45m 30s

1 Acetone to fix 5m



If slides keep falling off slide, air dry for 30 minutes before fixation

2 Water (wash)

3 Verhoeff's iron hematoxylin 20m

4 Water (3 washes)

5 Ferric chloride (2% wt/vol) 1m 30s

Water 3 washes

Check slide for differentiation at 1m30s and for every 30s afterward



* Halt staining when nuclei and elastin fibers are still black with weak background.

*** If over-differentiated, go back to the step 3.

6 Ethanol (95% vol/vol) 30s

7 Ethanol (100% vol/vol) 30s

8 Xylene 3m

9 Air dry uncovered 15m

10 Permount and coverslip