JUN 01, 2020

Verhoeff's Iron Hematoxylin Staining

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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.

ATTACHMENTS

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

OPEN ACCESS

DOI:

dx.doi.org/10.17504/protocols.io.be9sjh6e

External link:

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

Protocol Citation: Jeff Z. Chen, Deborah A. Howatt, Michael K. Franklin, Jessica J. Moorlegghen, Hisashi Sawada, Hong S. Lu, Alan Daugherty 2020. Verhoeff's Iron Hematoxylin Staining. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.be9sjh6e>

MANUSCRIPT CITATION:

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

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

Created: Apr 17, 2020

Last Modified: Jun 01, 2020

PROTOCOL integer ID:
35858

Keywords: aorta, elastin,
histology, verhoeff

MATERIALS

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 START INSTRUCTIONS

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

Note

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		
Note * 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