

Jun 02, 2020

Verhoeff's Iron Hematoxylin Staining

DOI

<https://dx.doi.org/10.17504/protocols.io.be9sjh6e>



Jeff Z. Chen¹, Deborah A. Howatt¹, Michael K. Franklin¹, Jessica J. Moorlegghen¹, Hisashi Sawada¹, Hong S. Lu¹, Alan Daugherty¹

¹University of Kentucky



Jeff Z Chen

University of Kentucky

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://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

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: April 18, 2020

Last Modified: June 02, 2020

Protocol Integer ID: 35858

Keywords: aorta, elastin, histology, verhoeff, quantification of elastin break, elastic tissue, iron hematoxylin, elastin break, elastic fiber, results in elastic fiber, tissue, elasin,

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 Hema...

255KB



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

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 Permunt and coverslip