

Oct 15, 2023

OSU TriState SenNet H&E staining of Formalin-Fixed, Paraffin-Embedded (FFPE) tissue sections

DOI

<https://dx.doi.org/10.17504/protocols.io.j8nlkombxv5r/v1>



Lorena Rosas¹, Ana L Mora¹, mauricio.rojas¹

¹Division of Pulmonary, Critical Care and Sleep Medicine, College of Medicine, Ohio State University, Columbus, Ohio, USA.

TriState SenNet

Cellular Senescence Net...



Lorena Rosas

The Ohio State University

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.j8nlkombxv5r/v1>

Protocol Citation: Lorena Rosas, Ana L Mora, mauricio.rojas 2023. OSU TriState SenNet H&E staining of Formalin-Fixed, Paraffin-Embedded (FFPE) tissue sections. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.j8nlkombxv5r/v1>

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: October 15, 2023

Last Modified: October 15, 2023

Protocol Integer ID: 89301

Keywords: H&E, FFPE, TriState, SenNET, tissue sections, staining, hematoxylin stains nucleic acid, stains protein, tissue sections hematoxylin, stain, different tissue type, tissue section, component dye, tissue, collagen, elastin in pink, protein, eosin

Funders Acknowledgements:

TriState SenNET (Lung and Heart) Tissue Map and Atlas consortium - NIH

Grant ID: Grant ID: U54AG075931

Abstract

Hematoxylin and eosin (H&E) stains are essential for recognizing the different tissue types and morphologic changes that contribute to the diagnosis of many diseases. The presence of two-component dye gives the advantage of differentially stains tissue components. Hematoxylin stains nucleic acid and ribosomes in blue, while eosin is stains proteins, such as collagen and elastin in pink. This protocol describes manual H&E staining of tissue sections from Formalin-Fixed, Paraffin-Embedded (FFPE) block.

Materials

⊗ Xylene **Fisher Scientific Catalog #X5P-1GAL**

⊗ 100% Ethanol **Fisher Scientific Catalog #22-032-601**

⊗ Hematoxylin solution according to Mayer **Merck MilliporeSigma (Sigma-Aldrich) Catalog #51275-500ML**

⊗ Acetic Acid 100% **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A6283**

⊗ Epredia™ Shandon™ Bluing Reagent **Fisher Scientific Catalog #6769001**

⊗ Eosin Y solution aqueous **Merck Catalog #HT110216-500ML**

⊗ Chemical Permunt Mounting Medium **Fisher Scientific Catalog #SP15-100**

⊗ Fisherbrand™ Superslip™ Cover Slips **Fisher Scientific Catalog #12-541-055**

⊗ Kimwipes **Kimberly-Clark Catalog #34120**

Equipment

BZ-X800

NAME

Microscope

TYPE

Keyence

BRAND

BZ-X800

SKU

Safety warnings

- ❗
 - Use universal safety precautions when handling tissue specimens and personal protective equipment (PPE).
 - All steps with flammable reagents, such as xylene/ethanol, must be done inside a fume hood.

Staining Procedure

1 Prepare slides

- 1.1 Place the slides at 60 °C for 30 minutes.
- 1.2 The solutions are fill in square glass staining jars.
- 1.3 Place slides in glass staining racks.

2 Deparaffinization of tissue slides: Remove remaining paraffin wax from tissues.

- 2.1 Place slides into a Xylene for 10 min, three times.
- 2.2 Remove Xylene and replace from ethanol (EtOH)
- 2.3 Place slides to 100% EtOH for 5 min, two times.

3 Rehydration of tissue slides: Remove alcohol from tissue and replace alcohol from water.

- 3.1 Place slides to 95% EtOH for 3 min, two times.
- 3.2 Place slides to 70% EtOH for 3 min, two times.
- 3.3 Place slides to Distilled H₂O (DI) for 1 min, two times.

4 Nuclear Stain: Hematoxylin is a basic dye, so it can bind and stain acid components of the cell such as nucleic acid and ribosomes.

4.1 Place slides to Mayer's Hematoxylin for 8 min.

4.2 Place slides to DI H₂O for 30 sec, two times.

4.3 Then rinse in tap water for 5 min.

5 Differentiation

5.1 Place slides to 5% Acetic Acid, 3 quick dips.

5.2 Place slides to DI H₂O for 30 sec, two times.

5.3 Then rinse in tap water for 3 min.

6 Bluing: Step important to give, cool bluish purple color to the acidic cellular components.

6.1 Place slides to Bluing Reagent for 2 min.

6.2 Then rinse in tap water for 3 min.

7 Counting staining: Is to give a pink color and this counterstain also helps to differentiate between nuclei and non-nuclear components in cells.

7.1 Place slides to Eosin Y solution aqueous for 2 min.

7.2 Place slides to DI H₂O for 30 sec, two times.

8 **Differentiation of eosin:** Removes unnecessary eosin – *check under microscope*.

8.1 Place slides to 95% EtOH for 3 min, two times.

9 **Dehydration of tissue slides:** Remove water with ascending concentration alcohol.

9.1 Place slides to 70% EtOH for 2 min, two times.

9.2 Place slides to 95% EtOH for 2 min, two times.

9.3 Place slides to 100% EtOH for 2 min, two times.

10 **Clearing:** To have clear background and to remove alcohol from tissue sections.

10.1 Place slides to Xylene for 2 min, two times.

10.2 Use *Kimwipe* to absorb some excess of Xylene. *"Do not allow the tissue section to dry out"*.

10.3 Add ~30uL of mounting medium on the tissue.

10.4 Place the coverslip, the medium will spread gradually to the edges of the coverslip. *"Avoid air bubbles underneath the cover slip"*.

Imaging



- 11 Slides are scanned using an EVOS™ M7000 microscope. Images are acquired with color brightfield using a 10X objective.