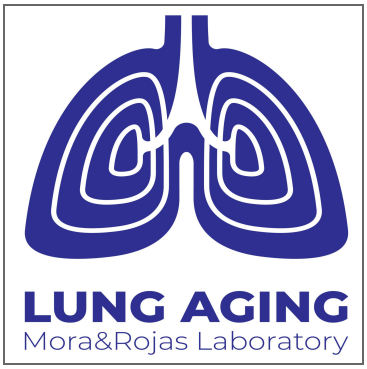

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Protocol status: Working
We use this protocol and it's working

Created: Oct 15, 2023

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

Lorena Rosas[1], Ana L Mora[1], mauricio.rojas[1]

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

Cellular Senescence Network (SenNet) Method Development Community

Lorena Rosas

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.

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Keywords: H&E, FFPE, TriState, SenNEt, tissue sections, staining

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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 Permount Mounting Medium **Fisher Scientific Catalog #SP15-100**
- Fisherbrand™ Superslip™ Cover Slips **Fisher Scientific Catalog #12-541-055**
- Kimwipes **Kimberly-Clark Catalog #34120**

Equipment

EVOS M7000 Imaging System	NAME
Microscope	TYPE
Invitrogen - Thermo Fisher	BRAND
AMF7000	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 H_2O (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 H_2O 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 H_2O 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 H_2O 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^{TM} M7000 microscope. Images are acquired with color brightfield using a 10X objective.