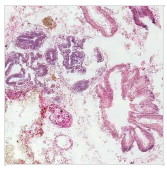

Version 2

Jan 29, 2021

Haematoxylin-Eosin stain for cryosections V.2

Simon Blanchoud[1]

[1]University of Fribourg

1 Works for me dx.doi.org/10.17504/protocols.io.brwxm7fn

Blanchoud lab, UNIFR

Marta Wawrzyniak
University of Fribourg

ABSTRACT

This protocol has been successfully used to stain our samples of colonial tunicates sectioned at 12 µm.

Adapted from Anna Jazwinska and Marguerite Kaczorowski.

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PROTOCOL CITATION

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KEYWORDS

colonial tunicates, Haematoxylin-Eosin, cryosections, histology

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GUIDELINES

Note that the solutions need to be filtered after dilution, which takes typically **30 min**.

MATERIALS TEXT

- Ammonium iron(III) sulfate [Iron alum] (CAS 7783-83-7)
- Sulfuric acid (CAS 7664-93-9)
- Hematoxylin (CAS 517-28-2)
- Eosin Y (CAS 17372-87-1) or Erythrosine B (CAS 16423-68-0; for a redder coloring).
- Glacial acetic acid
- Ethanol
- HCl
- H2O
- Histo-Clear II from National Diagnostics (HS-202) or Xylol
- Omnimount from National Diagnostics (HS-110).
- Coverslips
- Cellulose fliters

SAFETY WARNINGS

Note that Eosin solutions have to be discarded in the **Halogenated waste**.

Use Histo-Clear II which is a safer alternative to Xylol.

Groat's haematoxylin

1 Mix:
- **1 g** of Ammonium iron(III) sulfate
- **50 mL** of dH_2O
- **0.8 g** of sulfuric acid

1.1 Mix separately:
- **0.5 g** of hematoxylin dissolved in **50 mL** of EtOH (96%)

1.2 Pour the water-based mix into the EtOH one, filter if necessary.

0.1% HCl in 70% EtOH

2 Mix:
- **250 µl** of HCl ([M] **37 % (v/v)**)
- **100 mL** of EtOH ([M] **70 % (v/v)**)

Solution can be stored and used to differentiate ~30 slides.

Eosin Y 0.1%

3 Mix:
- **0.1 g** of Eosin Y
- **100 mL** of dH_2O
- **0.5 mL** of glacial acetic acid

Filter if necessary.

Histo-Clear II (HC)

4 Use Histo-Clear II for the final washes.

Citation: Simon Blanchoud (01/29/2021). Haematoxylin-Eosin stain for cryosections. https://dx.doi.org/10.17504/protocols.io.brwxm7fn

Staining protocol 35m 20s

5 Thaw for **00:20:00** or until no more signs of condensation. 20m

5.1 Rehydrate the cryosection in tap water **00:05:00** 5m

5.2 Stain in Haematoxylin **00:12:00** 12m

Haematoxylin solution can be reused many times and just needs to be filtered once in a while.

5.3 Differentiate each slide by shaking it vigorously in HCl-EtOH **00:00:20** 20s

5.4 Place in a coplin jar with tap water, place the jar in the sink and let tap water **run over it** for **00:10:00** 10m

5.5 Rinse in distilled water.

5.6 Stain in Eosin Y for **00:05:00** . 5m

5.7 Rinse in EtOH [M]**80 % (v/v)** (slides could be checked for their Eosin staining).

5.8 Start the wash “baths”: EtOH (80%, 100%, 100 %) & HC (I, II, III) **00:03:00** each. 3m

5.9 Mount under a coverslip using Omnimount.

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