



Haematoxylin-Eosin stain for cryosections V.1

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Works for me

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MATERIALS TEXT

Note that the solutions need to be filtered after dilution, which takes typically **30 min**.
Also note that Eosin solutions have to be discarded in the **Halogenated waste**.
Groat's haematoxylin solution can be reused many times and just needs to be filtered once in a while.

Groat's haematoxylin

- 1 Mix:
 - **1 g** of Ammonium iron(III) sulfate [Iron alum] (CAS 7783-83-7)
 - **50 mL** of dH₂O
 - **0.8 g** of sulfuric acid (CAS 7664-93-9)
- 1.1 Mix separately:
 - **0.5 g** of hematoxylin (CAS 517-28-2) 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 (**37 % (v/v)**)
 - **100 mL** of EtOH (**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 (CAS 17372-87-1)
 - **100 mL** of dH₂O
 - **0.5 mL** of glacial acetic acid

Filter.

Erythrosine B (CAS 16423-68-0) could be used instead of Eosin for a redder coloring.

Histo-Clear II (HC)

- 4 From National Diagnostics (HS-202). A safer alternative to Xylol for the final washes. Requires mounting with Omnimount from National Diagnostics (HS-110).

Staining protocol

35m 20s

5

- | | | | |
|-----|--|------------|-----|
| 5.1 | Thaw and rehydrate the cryosection in tap water | 🕒 00:05:00 | 5m |
| 5.2 | Stain in Haematoxylin | 🕒 00:12:00 | 12m |
| 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 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 using Omnimount