Hematoxylin and Eosin (H&E) Staining – Manual Protocol

(From Baylor College of Medicine)

Protocol for H&E Staining

•Place slides containing paraffin sections in a slide holder (glass or metal)

•Deparaffinize and rehydrate sections:

3 x 3′ Xylene *(blot excess xylene before going into ethanol)*
3 x 3′ 100% ethanol
1 x 3′ 95% ethanol 1 x 3′ 80%
ethanol
1 x 5′ deionized H2O

•While sections are in water, skim surface of hematoxalin with a Kimwipe to remove oxidized particles. Blot excess water from slide holder before going into hematoxalin.

•Hematoxalin staining:

1 x 3′ Hematoxalin
Rinse deionized water
1 x 5′ Tap water *(to allow stain to develop)* Dip 8-
12x (fast) Acid ethanol *(to destain)* Rinse 2 x 1′ Tap
water
Rinse 1 x 2′ Deionized water *(can leave overnight at this stage)*
(Blot excess water from slide holder before going into eosin)

(Last reviewed May 23rd, 2025)

•Eosin staining and dehydration:

1 x 30 sec Eosin *(up to 45 seconds for an older batch of eosin)*
3 x 5´ 95% ethanol
3 x 5´ 100% ethanol (*blot excess ethanol before going into xylene)* 3 x 15´ Xylene

•Coverslip slides using Permount (xylene based).

•Place a drop of Permount on the slide using a glass rod, taking care to leave no bubbles.

•Angle the coverslip and let fall gently onto the slide. Allow the Permount to spread beneath the coverslip, covering all the tissue.

•Dry overnight in the hood.

Reagents for H&E Staining

• **Acid Ethanol:** 1 ml concentrated HCl + 400 ml 70% ethanol

• **Hematoxylin:** Poly Scientific (Bayshore, NY) #s212A
Harris hematoxylin with glacial acetic acid

• **Eosin:** Poly Scientific (Bayshore, NY) #s176
Eosin Phloxine stain, working

• **Permount:** Fisher Scientific #SP15-100
Histological mounting medium