Hematoxylin and Eosin (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.

•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

•You can leave slides in xylene overnight to get good clearing of any water.

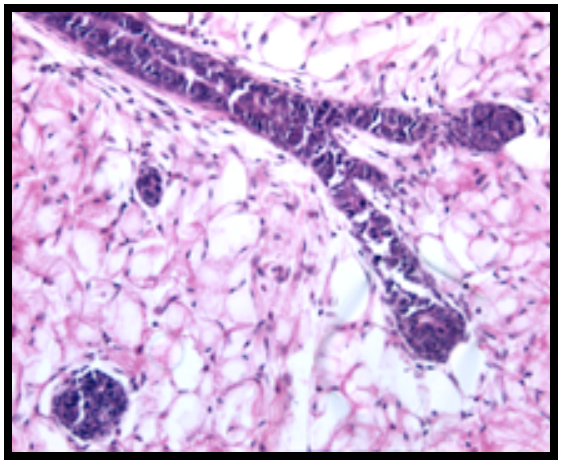
•Coverslip slides using Permount (xylene based).
•Place a drop of Permount on the slide using a glass rod, taking care to leave no bubbles.
•Angle the coverslip and let fall gently onto the slide. Allow the Permount to spread beneath the coverslip, covering all the tissue.
•Dry overnight in the hood.

Reagents for H&E staining:

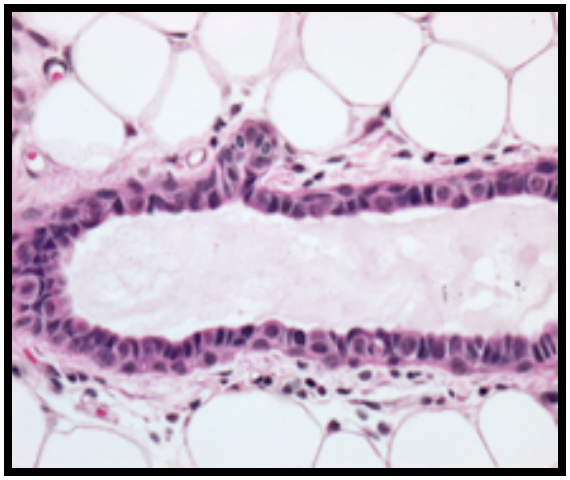
- **Xylene:** StatLab (Lewisville, TX) #8400
  Laboratory grade, Anapath brand
- **Acid Ethanol:** 1 ml concentrated HCl + 400 ml 70% ethanol
- **Hematoxylin:** Poly Scientific (Bayshore, NY) #s212A
  Harris hematoxylin with glacial acetic acid
- **Eosin:** Poly Scientific (Bayshore, NY) #s176
  Eosin Phloxine stain, working
- **Permount:** Fisher Scientific #SP15-100
  Histological mounting medium

Representative Images:

Mammary gland ducts (mature virgin)



20x magnification



40x magnification