



DISCOVERIES THAT MAKE A DIFFERENCE

### Core Imaging Facility

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## H&E Staining Protocol

*This procedure is performed with the slides in glass staining racks and the solutions in square glass staining jars/boxes.*

100% Xylene	3 minutes	**Begin here if you are using paraffin embedded sections.**
100% Xylene	3 minutes	
100% Xylene	3 minutes	
50:50 Xylene/100% EtOH	3 minutes	
100% EtOH	3 minutes	
100% EtOH	3 minutes	
95% EtOH	3 minutes	
95% EtOH	3 minutes	
Distilled H <sub>2</sub> O	2-3 rinses	**Begin here if you are using cryosections.**
Hematoxylin (Gill's 1X)	5 minutes	
Tap Water	Rinse slides under running tap water in staining box until the water is no longer colored. (~5 min.)	
Acid Alcohol (1%HCl in 70% ETOH)	Dunk 2-3 times until the sections turn pink	
Tap Water	Rinse 3-5 minutes	
Ammonia Water (1mL NH <sub>4</sub> OH in 1L H <sub>2</sub> O)	5-6 slow dunks; the sections should darken noticeably.	
Tap Water	Rinse 3-5 minutes	
Eosin Y	1 minute	

Tap Water	Rinse slides 3-4 times under running tap water.
95% EtOH	2 minutes
95% EtOH	2 minutes
95% EtOH	2 minutes
100% EtOH	2 minutes
100% EtOH	2 minutes
100% EtOH	2 minutes
50:50 Xylene/100% EtOH	2 minutes
100% Xylene	2 minutes
100% Xylene	2 minutes
100% Xylene	2 minutes

\*Keep slides in Xylene (no longer than one hour) until they are coverslipped.

Coverslip slides using Permount or a Xylene-based mounting medium of your choice.