



DISCOVERIES THAT MAKE A DIFFERENCE

## 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 Dunk 2-3 times until the sections turn pink (1%HCl in 70%ETOH)

Tap Water Rinse 3-5 minutes

Ammonia Water (0.25%) 5-6 slow dunks; the sections should darken noticeably. (1mL NH<sub>4</sub>OH in 1L H<sub>2</sub>O)

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.

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