

**STANDARD OPERATING PROCEDURE:  
HEMATOXYLIN and EOSIN (H&E) STAINING**

PI: _____	Room & Building: _____
Department: _____	Research Group: _____
Date: _____	Pertains to Lab Protocol: _____

**PROCEDURE**

Attach the experimental protocol(s) for hematoxylin and eosin staining.

**MATERIALS & HAZARDS**

Principal Materials Used	Corrosive	Irritant	Sensitizer	Reproductive toxin	Acutely Toxic	Carcinogen	Flammable	Combustible	Water-Reactive	Shock-Sensitive	Pyrophoric	Oxidizer	Biotoxin	Other Comments:
Xylene		X					X							See comment 1, below.
Ethanol (100, 95, 80%)		X					X							See comment 2, below.
Hydrochloric acid	X	X												See comment 3, below.
Ethanol (70%)		X					X							
Hematoxylin (with glacial acetic acid)	X	X												See comment 4, below.
Eosin		X												
Permout™		X					X							See comment 5, below.

- Other comments:**
1. May affect central nervous system and may be narcotic at high concentration. Keep away from heat, sparks, and open flame.
  2. May affect central nervous system.
  3. Hydrochloric acid is highly corrosive and causes severe burns on skin and eye contact and upon inhalation of vapor. Handle with care.
  4. May cause kidney damage. May cause central nervous system effects.
  5. Possible risk of harm to unborn child. May cause central nervous system depression. Aspiration hazard if swallowed.

**ENGINEERING/VENTILATION CONTROLS**

All parts of the H&E staining protocol should be performed in a chemical fume hood with the sash in the down position.

## RECOMMENDED PERSONAL PROTECTIVE EQUIPMENT

The level of skin and eye protection should be selected based on the potential for splashing and other forms of exposure.

### *Minimum potential for splash & exposure:*

- ✓ Chemical splash goggles
- ✓ Single pair of nitrile, PVC (vinyl), or neoprene gloves
  - Immediately replace with new gloves when splash occurs.
- ✓ Protective clothing (e.g. impervious lab coat, sleeves, closed-toed footwear)

### *When using or transferring large quantities or for spill clean-up:*

- ✓ Chemical splash goggles
- ✓ Face shield (if not working in a fume hood or if hood's sash is not in the down position)
- ✓ Double glove with nitrile, PVC (vinyl), or neoprene gloves
  - Immediately replace with new gloves when splash occurs.
- ✓ Chemical-resistant, impervious apron/smock/lab coat (PE or PVC) that ties in the back
  - Avoid using the traditional cotton-polyester white lab coat, which readily collects/absorbs compounds.
- ✓ Protective clothing (e.g. sleeves, impervious boots or PVC disposable shoe coverings, closed-toed footwear)

### *Additional precautions:*

- Always use the rack handles to submerge racks into the series of chambers containing xylene, ethanol, and aqueous solutions. Gloves are not suitable for immersion protection, only splash protection.

## MATERIAL SAFETY DATA SHEETS

MSDSs are available electronically via EHS Department's Web page:

<http://www.uos.harvard.edu/ehs/msds/>. An option, but consider collecting in a binder the MSDSs that arrive with each order.

## WASTE DISPOSAL

Refer to the *Laboratory Waste Guide* posted at

<http://www.uos.harvard.edu/ehs/longwood/HarvardLongwoodLabWasteGuide.pdf>

## EMERGENCY PROCEDURES

Refer to the emergency flip chart titled "*EHS Procedures and Response Guidelines*," posted in each laboratory and found on EHS's webpage at <http://www.uos.harvard.edu/ehs/longwood>

**ATTACHMENT**  
**HEMATOXYLIN AND EOSIN (H&E) STAINING PROCEDURE<sup>1</sup>**

- Place slides containing paraffin sections in a slide holder (glass or metal)
- Deparaffinize and rehydrate sections:

3 x 3 minutes	Xylene ( <i>blot excess xylene before going into ethanol</i> )
3 x 3'	100% ethanol
1 x 3'	95% ethanol
1 x 3'	80% ethanol
1 x 5'	deionized H <sub>2</sub> O
- 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'	<u>Tap water</u> ( <i>to allow stain to develop</i> )
Dip 8-12x (fast)	Acid ethanol ( <i>to destain</i> )
Rinse	2 x 1' Tap water
Rinse	1 x 2' Deionized water ( <i>can leave overnight at this stage</i> )
- Blot excess water from slide holder before going into eosin.
- Eosin staining and dehydration:

1 x 30 seconds	Eosin ( <i>up to 45 seconds for an older batch of eosin</i> )
3 x 5'	95% ethanol
3 x 5'	100% ethanol ( <i>blot excess ethanol before going into xylene</i> )
3 x 15'	Xylene
- You can leave slides in xylene overnight to get good clearing of any water.
- Coverslip slides using xylene-based Permount™.
- 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

*Reference:*

<sup>1</sup> Rosen lab website <http://www.bcm.edu/rosenlab>