Lab 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 |  |  |  |  |  |  |  |  |  |  |  |  |
| PermountTM |  | 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.

**REQUIRED PERSONAL PROTECTIVE EQUIPMENT**

(Refer to your lab’s PPE Assessment Report, supplemented with information here)

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.

**SAFETY REFERENCES**

Additional chemical safety information, including MSDSs and other information, is available electronically as tools at [ehs.harvard.edu/programs/safe-chemical-work-practices](http://ehs.harvard.edu/programs/safe-chemical-work-practices).

**WASTE DISPOSAL**

Refer to the *Laboratory Waste Guide* posted at [ehs.harvard.edu/node/7699](http://ehs.harvard.edu/node/7699).

**EMERGENCY PROCEDURES**

Refer to the [Emergency Response Guide](http://www.ehs.harvard.edu/programs/emergency-guidance) posted in your lab.

**ATTACHMENT 1**

HEMATOXYLIN AND EOSIN (H&E) STAINING PROCEDUREa

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

•Deparaffinize and rehydrate sections:

3 x 3 minutes 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 seconds 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 xylene-based PermountTM.

•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:***

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