

## Hematoxylin & Eosin Staining - Cryostat Sections

### I. Specimen:

Specimens include cryostat sections (cut at 4-6um), touch prints, or smears

### II. Materials & Reagents:

#### Equipment

Staining dishes with appropriate identification labels

Slide racks

Coverslips

Light microscope

#### Reagents

##### **XYLENE**

Chemical Formula: C<sub>8</sub>H<sub>10</sub> (p,m,o)

NFPA code: 2-3-0

**Hazards:** CARCINOGENIC, FLAMMABLE, mucous membrane irritant, good ventilation required

**Disposal:** Recycled; otherwise dispose of in 55 gallon waste drum for disposal by a licensed waste hauler

##### **ALCOHOL, 100%**

Chemical Formula: Alcohol mix (Ethanol, Methanol, & Isopropanol)

NFPA code: 1-4-0

**Hazards:** FLAMMABLE, Moderate skin or eye irritant, toxic by ingestion, could cause

blindness

**Disposal:** Recycled; otherwise dispose of in 55 gallon waste drum for disposal by a licensed waste hauler

##### **ALCOHOL, 95%**

Recycled alcohol **OR** add 50 ml deionized water to 950 ml Reagent Grade Alcohol (100%)

NFPA code: 1-4-0

**Hazards:** FLAMMABLE, Moderate skin or eye irritant, toxic by ingestion, could cause

blindness

**Disposal:** Recycled; otherwise dispose of in 55 gallon waste drum for disposal by a licensed

waste hauler

##### **HEMATOXYLIN (Gill III Formula)**

Source: Surgipath Catalog #01540

Chemical Formula: proprietary

NFPA code: 1-1-0

**Hazards:** Irritant, vapor is not hazardous under normal conditions of use.

**Disposal:** discarded down drain with large amounts of running water

##### **BLUING REAGENT**

Source: Richard Allan Catalog #7301 (distributed by Allegiance Healthcare)

Chemical Formula: proprietary

NFPA code: 0-0-0

**Hazards:** Not hazardous under normal conditions of use.

**Disposal:** discarded down drain with large amounts of running water

##### **EOSIN Y**

Source: Richard Allan Catalog #71204

Chemical Formula: proprietary

NFPA code: 1-3-0

**Hazards:** Flammable liquid, mild to severe skin irritant, moderate eye irritant

**Disposal:** discarded down drain with large amounts of running water

##### **PERMASLIP MOUNTING**

Chemical Formula: mixture of toluene & acrylic resin

NFPA code: 1-3-0

**MEDIA**

**Hazards:** Flammable liquid, vapor may be hazardous to health, use in well ventilated areas

**Disposal:** pour evenly over garbage in general waste

**Storage & Handling Requirements:**

**ALL CHEMICALS ARE STORED AT ROOM TEMPERATURE. WEAR GLOVES AND SAFETY GOGGLES WHENEVER POURING ANY CHEMICALS AND/OR STAINS.**

**III. Procedure:**

1. Fix slides in alcoholic formalin for 30 seconds (10% formalin or 95% alcohol can also be used).
2. Place fixed slides in staining rack.
  - Leave space between slides to allow a thorough rinsing to occur at each step.
  - Ensure that all slides in rack face forward to avoid inadvertently wiping off sections during coverslipping.
3. Rinse well in tap water.
  - Residual fixative should appear to “clear” off of slide when complete.
4. Stain nuclei in hematoxylin for 30 seconds.
  - Agitate rack gently if possible to enhance even staining.
5. Rinse well in tap water.
6. Blue slides in bluing reagent for 10 dips.
7. Rinse well in tap water (10 dips).
8. Counterstain in Eosin Y for 10 dips.
9. Differentiate with two changes of 95% alcohol for 10 dips each.
10. Dehydrate in three changes of 100% for 10 dips each.
11. Clear in two changes of xylene for 10 dips each.
12. Coverslip with a NON-aqueous mounting media.

**IV. Results & Interpretation:**

- Following specified procedures and adhering to the reagent maintenance schedule will result in well-stained H&E cryostat sections and overall quality patient care.

**V. Maintenance:**

1. Solutions are systematically maintained. Empty reagent reservoirs are carefully washed with warm, soapy water and dried thoroughly before use. Extra staining dishes are available so that cleaned dishes can dry overnight. Staining reservoirs are filled to level line ( $\approx$  250 ml). Reagents are rotated as follows:
  - **Hematoxylin & Eosin Y**  
The Hematoxylin and Eosin Y solutions are replaced weekly but fresh solution may be added as necessary to replace any evaporated solutions. Hematoxylin 7211 does not require filtering prior to use.
  - **Ammonia water**  
The ammonia water rinse is changed daily.
  - **95% Alcohols**  
The first 95% alcohol is discarded daily. The second 95% alcohol is rotated to the first position. Fresh 95% alcohol is placed in the second 95% alcohol position.

- **100% Alcohols**

The first absolute alcohol is discarded daily. The second & third absolute alcohols are rotated up respectively. Fresh 100% alcohol is placed in the third absolute alcohol position. Alcohols may be rotated up throughout the day if the frozen section volume is particularly high.

- **Xylene**

The first xylene is discarded daily. The second & third xylenes are rotated up respectively. Fresh xylene is placed in the last xylene position.

- **Tap water**

Tap water rinses are replaced after each staining batch.

2. Cover the reagent reservoirs with anti-evaporation lids when not in use. Wipe away any stains on metal rack using gauze and 95% alcohol.
3. Replace underpads and restock gauze, coverslips, pencils, and other disposables daily.
4. Remove stubborn stains with household bleach. Rinse dishes thoroughly prior to use.

## **VI. Calibrations:**

N/A

## **VII. Procedural Notes:**

1. A Quick Reference Guide for the H&E staining procedure is posted at each of the staining setups. See attachment 1.

## **VIII. References:**

Carson, Freida L., *Histotechnology - A Self Instructional Text*, Chicago, ASCP Press, 1990.

Crookham, Janet N. and Dapson, Richard W., *Hazardous Chemicals in the Histopathology Laboratory*, Battle Creek, ANATECH, LTD., 1991.

Richard Allan Medical Industries, *Customer Guidelines*, Richland, Richard Allan, Industries, 1991.

Sheehan, Dezna C., *Theory and Practice of Histotechnology*, St. Louis, C.V. Mosby Company, 1980.

### **Attachment 1**

