

**H&E Stain On Frozen Sections**

**Final Approval:** October 2010 **Effective:** October 2010

**Next Review Date:** August 2011

**List all stakeholder(s) and dates of approval:**

**Stakeholder Name(s):** Geoffrey Werner Date: 8.2010 Reviewed  Revised   
**Stakeholder Name(s):** Mark Magilner, MD Date: 5/2010 Reviewed  Revised   
**Stakeholder Name(s):** Date: Reviewed  Revised   
**Stakeholder Name(s):** Date: Reviewed  Revised   
**Stakeholder Name(s):** Date: Reviewed  Revised

**Describe briefly the most recent revision made to this policy, procedure or protocol & why:**  
New Procedure

**Purpose/Policy Statement:**

For diagnostic purposes, a RAPID nuclear & cytoplasmic staining technique is required to demonstrate relationships among cells and tissue components.

**Definitions:**

- N/A

**STEPS / KEY POINTS**

**SPECIMEN:** Tissue embedded in OCT medium is cut at 6 micron-thickness using a cryostat microtome and mounted on a glass microslide.

**MATERIALS, REAGENTS:**

Hematoxylin II- Richard-Allan #7231

Methyl-Formalin Fixative:  
Methyl alcohol . . . . . 720 ml  
37% Formaldehyde . . . 80 ml

**CAUTION – IRRITANT**  
Wear appropriate protective equipment.

100% Ethyl Alcohol (EtOH)

**CAUTION – FLAMMABLE, IRRITANT.**  
Wear appropriate protective equipment.

95% Ethyl Alcohol (EtOH)

**CAUTION – FLAMMABLE, IRRITANT.**  
Wear appropriate protective equipment.

70% Ethyl Alcohol (EtOH)

**CAUTION – FLAMMABLE, IRRITANT.**  
Wear appropriate protective equipment.

**CAUTION – FLAMMABLE, IRRITANT, SERIOUS HEALTH HAZARD, SUSPECTED CARCINOGEN.**  
Wear appropriate protective equipment.  
**CONTAINS FORMALDEHYDE.**  
Toxic by inhalation and if swallowed.  
Irritating to the eyes, respiratory system, and skin.  
May cause sensitization by inhalation or skin contact.  
Risk of serious damage to eyes.  
May cause cancer.  
Repeated or prolonged exposure increases the risk.

WORKING Phloxine Eosin  
95% EtOH . . . . . 3120 ml  
1% Eosin . . . . . 400 ml  
1% Phloxine B . . . . 40 ml  
Acetic Acid . . . . . 16 ml

CAUTION – FLAMMABLE LIQUID, IRRITANT,  
HEALTH HAZARD.

Wear appropriate protective equipment.

CAUTION – FLAMMABLE LIQUID,  
Wear appropriate protective equipment.

Cover glass

No.1 24 X 55

*CitriSolv* FisherBRAND - #22-14395

**INSTRUMENTATION OR EQUIPMENT:**

Frozen section H&E stain set.

CAUTION – FLAMMABLE LIQUID, IRRITANT.  
Wear appropriate protective equipment.

**PROCEDURE:**

1. Immediately immerse frozen-section slides in Methyl-Formalin for at least 30 sec.
2. Rinse in clean tap H<sub>2</sub>O for 10 dips.
3. Stain in Hematoxylin for 30 sec.
4. Rinse in clean tap H<sub>2</sub>O for 10 dips.
5. Blue in Scott's Tap Water for 5 sec.
6. Rinse in clean tap H<sub>2</sub>O for 10 dips.
7. 70% EtOH for 5 dips
8. Stain in Eosin for 20 sec.
9. 95% EtOH for 5 dips.
10. Absolute EtOH for 15 dips.
11. Absolute EtOH for 15 dips.
12. Citri-Solv for 15 dips.
13. Citri-Solv for 15 dips.
14. Mount with synthetic medium.

**CALCULATIONS:** N/A

**CALIBRATION:** N/A

**QUALITY CONTROL:**

1. Stains and reagents are filtered or changed daily and documented on a QA recordsheet.
2. Mounting medium consistency is validated on a daily basis. Reagent consistency must be balanced to avoid evaporation extremes following coverslipping. Either replace or add fresh mounting medium to the container. The mounting medium may also be "thinned": transfer the container to the Histology Lab., add small incremental amounts of Clearite III or xylene (reagents prohibited from the Pathology Gross Room), and mix thoroughly.

**RESULTS:** Nuclei - blue.  
Cytoplasm - pink.

**PROCEDURE NOTES:** NONE.

**LIMITATIONS OF PROCEDURE:** N/A

**REFERENCE:** A Manual for Histologic Technicians, Preece, 3rd ed., 1972. Little, Brown & Co., Boston. pp 226-234.

**MANUFACTURER PROCURE/INSERT:** NONE.

**DISTRIBUTION OF PROCEDURE:** Histology Policy & Procedure.

**Equipment/Supplies** (If Applicable):

Disposable gloves

Laboratory coat

Eye protection

Surgical mask or face shield if there is a possibility of specimen or chemical release into the air

**Form Name & Number or Attachment Name** (If Applicable):

surgpath09

**Author Position:**

Histology Supervisor

**Review/Revision Authority** (Position Not Individual Name):

Medical Director, Operation Manager, Histology Supervisor, Pathologist Assistant

**Expert Consultant Position/s** (Not Individual Name/s):

Pathology Physician Assistant

**References** (Required for Clinical Documents):

n/a

**Is there a Regulatory Requirement?** Yes  No

If yes, insert requirement information here:

**Review History** (No Changes):

n/a

**Revision History** (Note changes in area under header):

n/a

**Computer Search Words:**

n/a

**Policy, Procedure or Protocol Cross Reference Information:**

n/a