FROZEN SECTIONS PROTOCOL

Principle
Enclosed are instructions for how to integrate the following Ultralight Histology products into existing laboratory protocols. These instructions are specifically for frozen sections.
• CRET
• Cryostat chuck, heat sink, & heat reservoir

Disclaimer
ANY DEVIATION FROM THE FOLLOWING INSTRUCTIONS WILL GREATLY DIMINISH THE QUALITY OF THE FINISHED SLIDE. ALL STEPS MUST BE ADHERED TO STRICTLY.

MSDS
CRET consists of mixed polymers of amino acids. These substances are non-hazardous (edible) and used in micro-molar amounts. For full MSDS information, please visit the Mercedes Medical website.

Validation
The tissue detail achieved with the Ultralight Histology process has been validated to published electron microscopy images at 1000X.
CRYOSTAT TOOLS

Principle
Frozen section examinations are performed for the rapid diagnosis of material obtained by surgical biopsy. Instead of paraffin embedding, the tissue is rapidly frozen to allow cutting of thin sections. The tissue is then stained with hematoxylin and eosin and examined under a microscope.

Specimen Requirements
Fresh, unfixed surgical tissue or biopsy specimen(s).

Equipment
Copper heat sink
Cryostat Chucks
Coated block of solid copper

Tissue-Tek Cryomold
Tissue Tek OCT Compound

Quality Control
The microtome in the cyrostat is lubricated with special low temperature oil. Remove any tissue fragments from the cryostat. Check the cryostat microtome blade for sharpness. Disposal blades are used. Temperature is monitored, plus or minus five degrees of -21˚C.
CRYOSTAT PROCEDURE

Procedure
1. Store the chrome plated copper heat sink on the handle in the coldest part of the cryostat prior to use. The heat sink base hole should be in the middle beneath the cryostat chucks that are to be used. The ultra-light mounting chucks have cross-hatched grooves cut into the face to help hold the mounting media and frozen tissue. Both the heat sink and mounting chucks are to be used in performing frozen sections.

2. Prior to use, apply a circular portion of the OCT to the mounting chuck and allow it to freeze in the cryostat, taking care not to allow bubbles to form.

3. When ready to freeze unfixed tissue, remove a mounting chuck from the cryostat, add a small amount of additional room temperature OCT.

4. Place the tissue to be frozen onto the new OCT.

5. Replace the mounting chuck into the solid copper heat sink.

6. Surround the tissue with more fresh OCT and immediately, lightly apply a coverslip to the surface and then apply the face of the heat sink on a handle, lightly to the surface. After approximately 10 seconds, the freezing will be sufficient to support the weight of the heat sink. This step will require practice to perfect to where the tissue is not reoriented or squeezed out by the weight of the heat sink.

7. The heat sink remains on the face of the tissue for a minute or more, this will extract all the heat from the tissue and prevent ice crystals (ice artifacts) from forming.

8. Perform routine H&E staining. We recommend using the CRET reagent (4 drops per 500mL of hematoxylin and eosin) to increase the saturation of the stains.
Description
Pristine™ is designed to mediate the degradative effects of formaldehyde. Frozen sections are not fixed in formalin but frozen and therefore, Pristine is not a necessary product in the protocol for frozen sections.
CRET

**Description**
For use in histology staining protocols, for both permanents and frozen sections. CRET (Color Resonance Energy Transfer) acts as a dial-in color adjuster (saturation) for stains allowing them to customized to preference.

**Mercedes Medical Item Code:** TBA, 15mL

**Storage & Shelf Life**
Refrigerate for best results. Shelf life is 6 months.

**Procedure (Permanents & Frozens)**

For Initial Use

- Add 4 drops per 500mL of hematoxylin
- Add 4 drops per 500mL of eosin

For On-going Maintenance
At approximately 500 slides or when stains would ordinarily be changed.

- Add 2-4 drops per 500mL of hematoxylin
- Add 2-4 drops per 500mL of eosin

Mix well after each addition.

**Note**
Whenever one or both of the stains begin to pale, follow the above steps for on-going maintenance.
## PRODUCT DETAILS

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<th>Item:</th>
<th>Mercedes Medical ID code:</th>
<th>Storage Requirements:</th>
<th>Expiration</th>
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Recommendations for oscillating table:  
Barnstead Lab Line MaxQ 2000  
SHKA2000