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KAPP-Sen TMC: Routine H&E Processing and Staining using Leica ST5010 AutoStainer

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We use this protocol and it's working

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Abstract

This protocol details steps for tissue handling and sectioning, routine H&E staining using the Leica ST5010 AutoStainer XL, and imaging using the NanoZoomer-SQ digital slide scanner. This procedure applies to the members of the Single Cell Biology lab in Farmington, CT.

MATERIALS

1

NAME	VENDOR	CATALOG #
Acrytol Mounting Media	Leica	3801720
<i>Chemical Fume Hood</i>	-	-
<i>Corning™ LSE Mini Standard Incubator, 9.2 L</i>	Thermo	07-201-060
Deionized Water	-	-
Disposable Transfer Pipettes	VWR	10754-268
<i>Epredia™ HM 355S Fully-Automated Microtome</i>	Cardinal Health	905200
Epredia™ Shandon™ Bluing Reagent	Thermo	6769001
Ethanol, Absolute (200 Proof), Molecular Biology Grade	Thermo	BP28184
Glass coverslip (1mm, 24 x 50)	Thermo	NC0106068
Hematoxylin Solution, Mayer's	Sigma	MHS32-1L
Leica Brush with Magnet	Leica	NC0773452
<i>Leica ST5010 AutoStainer XL</i>	Leica	14 0456 35340
ST5010XL Accessory kit	Leica	0456 35660
Low-Profile Disposable Blades DB80LX	Leica	14035843496
Micro-dissecting Forceps	Sigma	F4142-1EA
<i>NanoZoomer-SQ digital slide scanner</i>	Hamamatsu	C13140-01
NPD.view software	Hamamatsu	-
RNase Zap™ RNase Decontamination Solution	Invitrogen	AM9780
Round Camel Hair Brush No. 6	JBC and Co	904-5
VWR® Premium Superfrost® Plus Microscope Slides	VWR	48311-703
Xylene, Reagent Grade	Sigma	214736-4L

DEFINITIONS AND ACRONYMS

- 2 **FFPE:** Formalin-Fixed, Paraffin-Embedded
H&E stain: Hematoxylin and Eosin stain
SCBL: Single Cell Biology Lab

KEY POINTS

3 5.1 Safety Key Points:

5.1.1 Conduct all slide cover-slipping within a fume hood when using xylene-based mountant.

5.1.2 Carefully dispose used microtome blades in the appropriate sharps container.

5.1.3 Wear all appropriate PPE (e.g. gloves, goggles, lab coat, etc)

5.2 Quality Key Points:

5.2.1 Allow FFPE tissue sections to expand appropriately in the water bath to prevent folds and wrinkles.

5.2.2 Minimize RNase contamination by spraying all microtome components, forceps, and brushes with RNase Zap before processing.

PROCEDURE

4 6.1 Tissue Preparation and Sectioning

6.1.1 Wear nitrile gloves cleaned with RNase Zap spray. Using RNase Zap spray, clean the following items in the workspace: microtome (including any surfaces that may come into contact with blocks or your hands, such as the block holder, knife holder, handle, etc), all forceps and brushes, the ice tray, inside of mini incubator, and the glass Pyrex water bath insert.

6.1.2 Thoroughly rinse the glass insert with deionized water. Fill with deionized water and heat as usual to a final temperature of 40°C.

NOTE: Failure to rinse the glass insert will cause static repulsion, making it very difficult to place tissue on a slide correctly.

6.1.3 Set the LSE mini incubator to 40°C. Monitor internal temperature with a thermometer until it reaches the desired temperature.

6.1.4 Blocks will arrive wrapped in aluminum foil to protect from light and should be stored in a refrigerator at 4°C. Once the workspace is ready, remove the blocks from the refrigerator, remove the aluminum foil, and place the block with the tissue facing down in a clean ice tray containing wet ice. Hydrate the blocks for approximately 20 minutes before sectioning.

6.1.5 Wearing nitrile gloves cleaned with RNase Zap spray, collect sections as follows:

6.1.5-A If applicable, collect any curls for RNA extraction (2×10µm curls; remove extra paraffin if possible). Store at 4 °C, protected from light, until ready to process. Label all tubes with SCBL curl IDs in correspondence to each unique sample provided by the customer (e.g., CU250001 – Sample Name 1).



6.1.5-B Using the water bath, forceps and brushes prepared as per 6.1.1, begin to section each block at 5µm. Discard the first two sections before taking a ribbon, to prevent any RNase contamination. Place the ribbon into the water bath, then collect sections on pre-labeled Superfrost slides containing the SCBL slide ID corresponding to each unique sample provided by the customer (e.g., SL250001 – Sample Name 1).

6.1.5-C Repeat steps 6.1.5-A and 6.1.5-B for the remaining blocks and transfer all unstained slides to a Leica slide rack. 6.1.5-C1 NOTE: It is extremely important to change blades and clean the workstation and accessories with RNase Zap spray between samples.

6.1.6-D Once all sectioning is complete, transfer the rack to the mini incubator to dry for 1 hour at 40 °C. Ensure the slides within the rack remain vertical to prevent water bubbles from forming under the paraffin.

5 **6.2 Routine H&E Protocol: Leica AutoStainer XL**

6.2.1 Create the following staining protocol on the Leica ST5010 AutoStainer XL:

6.2.1-A



Station	Reagent	Time (min:sec)	Exact	Dip
Oven	Oven 58°C	30:00	N	N
1	Xylene	04:00	N	Y
2	Xylene	03:00	N	Y
3	Xylene	03:00	N	Y
4	100%	02:30	N	Y
5	100%	02:30	N	Y
6	95%	02:30	N	Y
7	70%	02:30	N	Y
8	50%	02:30	N	Y
W3	Wash	01:00	N	N
9	Mayer's Hematoxylin	04:00	Y	Y
W4	Wash	02:00	N	N
12	Bluing Buffer	01:00	Y	Y
W5	Wash	01:00	N	N
13	Eosin	00:45	Y	Y
14	95%	02:00	Y	Y
15	95%	02:00	N	Y
16	100%	02:00	N	Y
17	100%	02:00	N	Y
18	Xylene	02:00	N	Y
19	Xylene - Exit	02:00	N	Y

NOTE: Washes are done using DI water. The final xylene is an exit station, meaning the tissues will hold at that station indefinitely. Slides should not wait in the exit station for more than 1 hour before cover-slipping.

6.2.1-B Prepare each station with its appropriate reagent. Make sure the oven has been set to 58 °C and is at the appropriate temperature before continuing.

6.2.2 Remove the slide rack from the mini incubator after the slides have been dried appropriately and transfer it to the loading position in the autostainer. Choose the protocol created from 6.2.1-A and press the "Load" button to begin the process.

6.2.3 After the run is complete, remove the final xylene/exit station from the autostainer and place it inside a fume hood for cover-slipping. Discard any reagents, if necessary, from the autostainer.

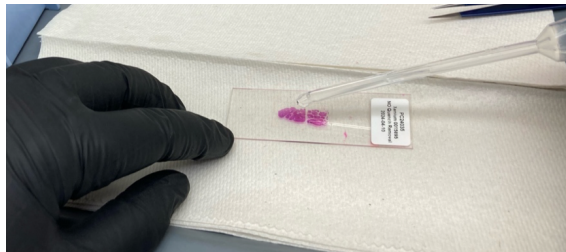
6 6.3 Post-H&E Cover-Slipping

6.3.1 Perform all subsequent steps inside a fume hood. Prepare the workspace with paper towels, and gather the following materials: Acrytol, glass coverslips, disposable transfer pipette, and forceps.

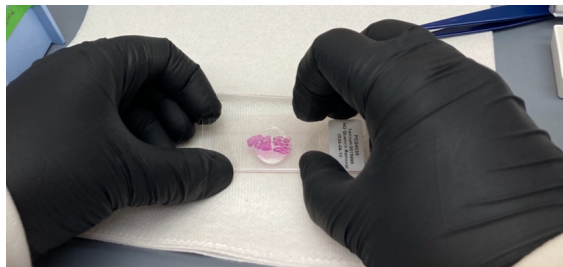
6.3.2 Remove slides from 6.2.3 one at a time onto a paper towel inside the fume hood. Leave the remaining slides in the xylene vessel to prevent the tissue from over-drying.

NOTE: If the tissue is dried before cover-slipping, there is a higher chance of artifacts such as hematoxylin oxidation and inadequate dispersion of mountant across the slide.

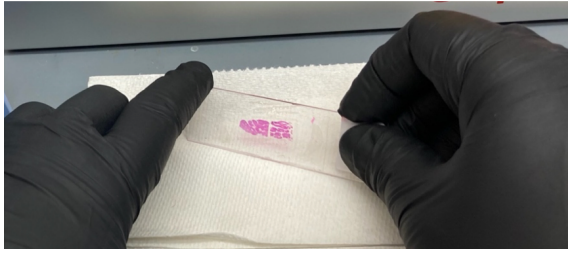
6.3.3 Use a disposable transfer pipette to add 2-3 drops of Acrytol mounting media along the long edge of the slide.



6.3.4 Quickly remove any dust particles from the glass coverslip. Place the long edge of the coverslip on the edge of the slide closest to you, tip the slide towards yourself so the mountant begins to reach the coverslip, and gently push down until the mounting media has spread evenly across the slide area containing tissue.



6.3.5 Remove excess mountant by placing the edges along the paper towel and carefully moving it in a pivot motion. Ensure mountant does not spread over the top of the coverslip.



6.3.6 Gently press down on the coverslip to remove any bubbles that remain above the stained tissue.

NOTE: Bubbles near the edges of the slide can remain if it does not cover any tissue areas.

6.3.7 Repeat steps 6.3.2–6.3.6 with the remaining slides. Allow slides to fully cure in the fume hood for at least 1 hour before imaging. Mounted and cured slides can be stored at room temperature.

7 **6.4 Slide Scanning**

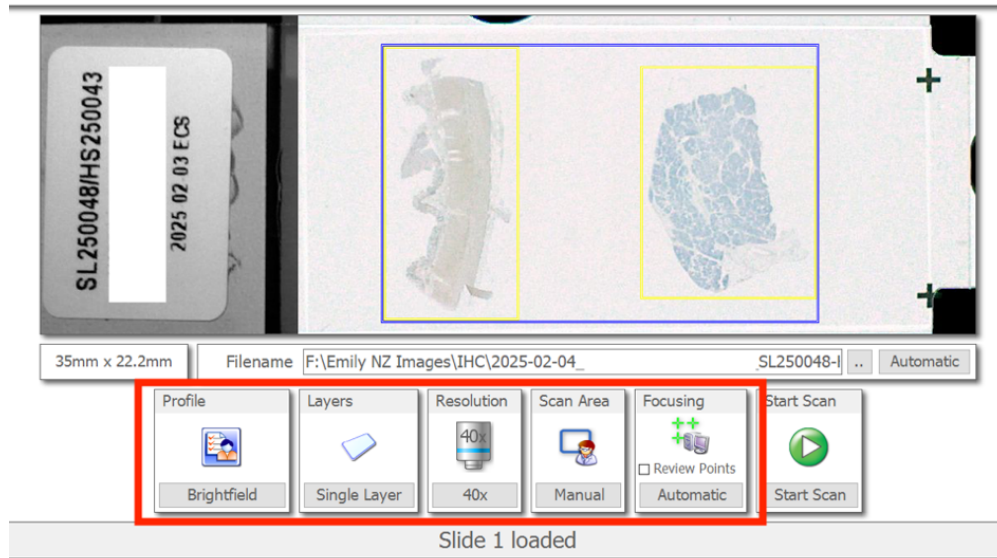
6.4.1 Turn on the NanoZoomer SQ and launch the NDP.scan SQ software. Start a new job using the “manual” setting.

6.4.2 Clean the top and bottom of the slide to remove any dust particles, then load the slide into the instrument.

6.4.3 Drag and select the region of interest for the tissue in the slide overview at the top of the interface. Refer to the image in 6.4.3-A for the appropriate settings, shown in the red box.

6.4.3-A

Manual Mode



6.4.4 Press "Start Scan" and allow the software to autofocus the tissue. Depending on the size and complexity of the tissue, the scan can take up to 20 minutes before completion.

6.4.5 Once the scan is complete, press "Load Next Slide" and follow the same process referring to steps 6.4.2-6.4.4 for all remaining slides. Store slides at room temperature, protected from light.

6.4.6 Review all images in the NDP.view software to check for out of focus regions or staining artifacts.