Histology Core Facility, University of Saskatchewan
STAINING SOP

Staining Station Instructions:

- Make arrangements for training with Adi prior to using the staining station for the first time
- Please ensure that during your orientation, you understand both the staining procedure and how to maintain the staining set-up
- If back up/replacement of solution/s is needed, it is best to do that just before you start staining. **Talk to Adi about this**
- **You must book a slot for staining online at least 24 H in advance and check with Adi about the filtration**
- Make sure that the fume adsorber is turned on before you start the process of staining
- You can stain up to 24 slides at a time using the slide holder
- Please record the number of slides and racks of slides (1-24 slides in 1 rack) that have been stained in the log book found just above the staining station. This information is needed to keep track of usage to invoice and also check status of reagents that may need changing.
- To prevent excessive carryover of reagent from one staining jar to the next, please drain back as much reagent as possible
- When performing H&E staining, Harris Hematoxylin must be filtered before use or you may end up with stain precipitates on your finished slides
- Eosin does not have to be filtered as frequently
- **Please leave this staining station in the condition that you'd like others to leave it for you!**
- For staining fees per slide, refer to cost recovery pricelist on the histology core facility website
- **The fume adsorber must be left turned on for at least 3 hours after staining. If working after hours or on weekends, leave the fume adsorber turned on overnight or over the weekend.**

Coverslipping Station Instructions:

- The cost of coverslipping is included in the overall cost of staining for one slide
- First time users must seek training for coverslipping from Adi
- Make sure that the fume adsorber is turned on before you start the process of coverslipping
- If you are running low on mounting media, ask Adi or alternatively transfer Surgipath MM24 mounting media (Leica Inc.). This can be found in the cabinet above the coverslipping station with a label on the door indicating mounting media
- We provide a variety of sizes of coverslips. **Please use the long coverslips (24x50 mm or 24 x 60 mm) only if you have those many sections on a slide requiring coverslipping**
- If you run out of coverslips , you can grab a box of coverslips from the drawer labelled coverslip (below the knife sharpener)

H & E Staining Protocol:

1.	3 minutes:	Xylene – 1
2.	3 minutes:	Xylene – 2
3.	1 minute:	Xylene / Absolute Alcohol
4.	1 minute:	Absolute Alcohol – 1
5.	1 minute:	Absolute Alcohol – 2
6.	1 minute:	95 % Ethanol
7.	1 minute:	Tap Water – WHITE BUCKET
8.	Rinse:	Distilled Water – BLUE BUCKET
9.	0.5 - 5 minutes:	Harris Hematoxylin **(Duration can vary with type of tissue)**
10.	Wash:	Tap Water (several changes) – WHITE BUCKET
9.	2 dips:	Acid Alcohol (0.5% HCl in 95% Ethanol)
10.	Wash:	Tap Water (several changes) – WHITE BUCKET
11.	5 seconds:	Saturated Aqueous Lithium Carbonate
12.	3 minutes:	Running Tap Water – WHITE BUCKET
13.	Rinse:	Distilled Water – BLUE BUCKET
14.	1 minute:	Eosin
15.	1 minute	70 % Ethanol
16.	1 minute:	95 % Ethanol
17.	1 minute:	Absolute Alcohol – 1
18.	1 minute:	Absolute Alcohol – 2
19.	1 minute:	Absolute Alcohol / Xylene
20.	1 minute:	Xylene – 1
21.	1 minute:	Xylene – 2
22.	1 minute:	Xylene – 3
23.	1 minute:	Xylene – 4

Leave slides in xylene until ready to coverslip; **don't let them dry out!**

Coverslip slides in a permanent mounting medium in the Coverslipping station only!!!.

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