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**
- **Working Wiegert's Hematoxylin has a shelf life of up to 1 month. Ensure that you check if Wiegert's is available for this staining protocol**
- 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
- **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

Safranin-O Staining Protocol:

1.	4 minutes:	Xylene – 1
2.	4 minutes:	Xylene – 2
3.	4 minutes:	Xylene – 3
3.	1 minute:	Xylene / Absolute Alcohol
5.	1 minute:	Absolute Alcohol – 1
6.	1 minute:	95 % Ethanol – 1
7.	1 minute:	95 % Ethanol – 2
7.	1 minute:	70 % Ethanol
8.	1 minute	Tap Water – WHITE BUCKET
9.	Rinse:	Distilled Water – BLUE BUCKET
10.	10 minutes:	Wiegert's Hematoxylin working solution (good for up to 1 month)
11.	10 minutes:	Running Tap Water – WHITE BUCKET
12.	Rinse:	Distilled Water – BLUE BUCKET
13.	5 minutes	Fast Green
14.	10-15 seconds:	1% Acetic Acid solution
15.	5 minutes	Safranin-O
16.	1 minute	70 % Ethanol
17.	1 minute:	95 % Ethanol
18.	5 minute:	Absolute Alcohol – 1
19.	5 minute:	Absolute Alcohol – 2
20.	5 minute:	Absolute Alcohol – 3
26.	5 minute:	Absolute Alcohol / Xylene
27.	1 minute:	Xylene – 1
28.	1 minute:	Xylene – 2
29.	1 minute:	Xylene – 3

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!!!.

Nuclei – Black

Cytoplasm – Bluish green

Cartilage, Musin, Mast cell granules – orange to red

This protocol is modified from Schmitz et al. 2010 (https://doi.org/10.1016/j.joca.2010.05.026)