UNIVERSITY OF SASKATCHEWAN
Health Sciences
HEALTHSCIENCES.USASK.CA

HISTOLOGY CORE FACILITY
B109B Health Sciences Building | 107 Wiggins Road
Saskatoon SK S7N 5E5 Canada
Email: hsbhistology@usask.ca | Telephone: 306-966-4095
healthsciences.usask.ca/histology

Staining Station Instructions:

- Make arrangements for training with Shreyas 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 Shreyas about this.**
- **You must book a slot for staining using the booking calendar at least 24 H in advance.**
- 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 logbook found above the weighing scales. This information is needed to keep track of usage and check status of reagents.
- To prevent excessive carryover of reagent from one staining jar to the next, please drain back as much reagent as possible.
- 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 Shreyas.
- Make sure that the fume adsorber is turned on before you start the process of coverslipping.

October 2023

- If you are running low on mounting media, ask Shreyas or alternatively transfer the Acrytol 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 (e.g., 24x50 mm or 24x60 mm) only if you have those many sections on a slide requiring coverslipping**.
- **AFTER COVERSLIPPING, SLIDES MUST BE PLACED IN THE FUMEHOOD OVERNIGHT FOR DRYING OUT! THE MOUNTING MEDIA CONTAINS XYLENE AND CAN RELEASE NOXIOUS FUMES - NO EXCEPTIONS!!!!**
- If you run out of coverslips, you can grab a box of coverslips from the drawer labelled coverslip (below IHC-2 work bench and opposite to the microwave.

Alcian Blue and Nuclear Fast Red Staining Protocol for Paraffin Embedded Tissues:

Slides are warmed to room temperature for at least 1 hour to get them to room temperature. After slides are thawed at room temperature, they are baked at 60 °C for at least one hour or overnight prior to the next step i.e., deparaffinization. The slide baking can be conducted in Oven#1 (close to the main lab entrance).

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.	30 Minutes	Alcian Blue solution
10.	Wash	Tap Water (2-3 changes) – WHITE BUCKET
11.	2 Minutes	Running Tap Water – WHITE BUCKET
12.	Rinse	Distilled Water – BLUE BUCKET
13.	5 Minutes	Nuclear Fast Red Solution
14.	1 Minute	Running Tap Water – WHITE BUCKET
15.	3 Minutes	95% Ethanol
16.	3 Minutes	Absolute Alcohol – 1
17.	3 Minutes	Absolute Alcohol – 2
18.	1 Minute	Absolute Alcohol / Xylene
19.	1 Minute	Xylene – 1
20.	1 Minute	Xylene – 2
21.	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! **AFTER COVERSLIPPING, SLIDES MUST BE PLACED IN THE FUMEHOOD OVERNIGHT FOR DRYING OUT! THE MOUNTING MEDIA CONTAINS XYLENE AND CAN RELEASE NOXIOUS FUMES - NO EXCEPTIONS!!!!**

Results:

Strongly acidic sulfated mucosubstances (goblet cells of intestine) ---------- blue

Nuclei ---------- pink to red

Cytoplasm ---------- pale pink

Reagents:

3% Acetic Acid Solution:

Glacial acetic acid ----------3 ml

Distilled water----------97 ml

Alcian Blue Solution (pH 2.5):

Alcian blue, 8GX ---------- 1 g

Acetic acid, 3% solution---------- 100 ml

Mix well and adjust pH to 2.5 using acetic acid.

0.1% Nuclear Fast Red Solution:

Nuclear fast red ---------- 0.1 g

Aluminum sulfate---------- 5 g

Distilled water ----------100 ml

Dissolve aluminum sulfate in water. Add nuclear fast red and slowly heat to boil and cool. Filter and add a grain of thymol as a preservative.

Revisions:

October 2023- The protocol was updated from the references below for the histology core facility by Shreyas Jois and Adi Manek with facility specifics.

October 2023

References:

1. **https://healthsciences.usask.ca/facility-services/Histology/alcian-blue-and-alizarin-red-s_ffpe-tissues-version-0.1-july-2022.pdf**
2. **http://www.ihcworld.com/_protocols/special_stains/alcian_blue.htm**
3. **https://www.ihcworld.com/_protocols/counterstain_solutions/nuclear_fast_red.htm**