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.

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- 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.

Methenamine silver plating staining (Gomori method) for Paraffin Embedded Tissues:

(Using Sigma-Aldrich kit 1.00820)

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).

Do not use metal tweezers and do not allow any other metal objects to come into contact with the slides. The stated times should be adhered to guarantee an optimal staining result.

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.	2 Minutes	Distilled water – BLUE BUCKET
8.	10 min	Reagent 1 (Periodic acid solution)
9.	30 sec	Distilled water (3 changes) – BLUE BUCKET
10.	35 - 45 min	Freshly prepared silver nitrate/methenamine borate solution at 55 - 57 °C*
11.	30 sec	Distilled water (3 changes) – BLUE BUCKET
12.	1 min	Reagent 4 (Gold chloride solution)
13.	30 sec	Distilled water – BLUE BUCKET
14.	2 min	Reagent 5 (Sodium thiosulfate solution)
15.	3 min	Running tap water WHITE BUCKET
16.	30 sec	Distilled water
17.	2 - 3 min	Reagent 6 (Light green SF solution)
18.	30 sec	Distilled water – BLUE BUCKET
19.	1 min	Ethanol 70 %

20. 1 min Ethanol 95 %
21. 1 min Absolute Alcohol – 1
22. 1 min Absolute Alcohol – 2
23. 5 min Xylene – 1
24. 5 min Xylene – 2

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!!!!**

The use of immersion oil is recommended for the analysis of stained slides with a microscopic magnification >40x.

Results:

Fungal elements ----------dark brown to black

Basal membranes ----------dark brown to black

Background ----------green

Reagents:

*Place the silver nitrate/methenamine borate solution together with the sample to be stained into the water bath previously heated to 55 - 57 °C, maintain this temperature throughout the staining process and stain for

35 - 45 minutes until achieving the desired intensity.

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.

References:

1. https://healthsciences.usask.ca/facility-services/Histology/pas-staining-protocol-ffpe-tissues-version-1.0-january-2023.pdf
2. https://www.sigmaaldrich.com/deepweb/assets/sigmaaldrich/product/documents/343/612/100820-en.pdf