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 and check with Shreyas about the filtering of Hematoxylin**
- Before staining with Harris Hematoxylin, it must be filtered before use, or you may end up with stain precipitates on your finished slides
- 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 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
- **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. 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
- If you are running low on mounting media, ask Shreyas 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 (e.g. 24x50 mm or 24 x 60 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 work bench and opposite the microwave

## Instructions for using Schiff's reagent:

- Schiff's reagent is stored in the dark at 4ºC and keep the solution out only for the time required during the staining procedure.
- **DO NOT** return used Schiff's reagent to the stock bottle, refrigerate in the designated Used Schiff's Reagent bottle.
- **Schiff's reagent is light sensitive. Minimize exposure to light when you get to this step.**
- The solution can be used until it remains colorless, discard when pinkish color develops.

# Periodic Acid-Schiff (PAS) Staining Protocol For Paraffin Embedded Tissue sections:

| | | |
|---|---|---|
| 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. | 5 minutes: | 1% Periodic Acid **(Varies from 3 to 10 min)** |
| 10. | 5 minutes: | Running Tap Water – WHITE BUCKET |
| 11. | Rinse: | Distilled Water – BLUE BUCKET |
| 12. | 15 minutes: | Schiff's Reagent (varies from 10 to 30 min) – (LIGHT SENSITIVE) |
| 13. | 5 minutes: | Running Tap Water – WHITE BUCKET |
| 14. | Rinse: | Distilled Water – BLUE BUCKET |
| 15. | 3 to 5 minutes | Harris Hematoxylin |
| 16. | Wash: | Tap Water (2-3 changes) – WHITE BUCKET |
| 17. | 2 dips: | Acid Alcohol |
| 18. | Wash: | Tap Water (2-3 changes) – WHITE BUCKET |
| 19. | 15 seconds: | Saturated Aqueous Lithium Carbonate |
| 20. | 3 minutes: | Running Tap Water – WHITE BUCKET |
| 21. | Rinse: | Distilled Water – BLUE BUCKET |

**NO EOSIN COUNTERSTAIN**

| | | |
|---|---|---|
| 22. | 1 minute | 70 % Ethanol |
| 23. | 1 minute: | 95 % Ethanol |
| 24. | 1 minute: | Absolute Alcohol – 1 |
| 25. | 1 minute: | Absolute Alcohol – 2 |
| 26. | 1 minute: | Absolute Alcohol / Xylene |
| 27. | 1 minute: | Xylene – 1 |
| 28. | 1 minute: | Xylene – 2 |

29. 1 minute: Xylene – 3
30. 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!!!. **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!!!!**

**Revisions:**

1. Based on H&E STAINING PROTOCOL FFPE Tissue Version 2.0 September 2021 by Adi Manek
2. January 2023- Updated for facility by Shreyas Jois and Adi Manek with facility specifics