



H&E Staining of cryopreserved samples, Instructions:

Before you stain for the first time:

- Plan for training with Shreyas prior to using the staining station for the first time
- **Frozen slides need to be thawed at room temperature overnight or at least for 1 H prior to staining**
- **For tissue fixation, we provide 10% NBF and paraformaldehyde (PFA) powder (4% PFA to be prepared by yourself). If you need to fix your cryo-sections before staining, talk to Shreyas and discuss the process.**
- **You must book a slot for staining using the booking calendar at least 24 H in advance**

Preparation list

- If back up/replacement of solution/s is needed, **talk to Shreyas about this**
- 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 above the weighing scales. This information is needed to invoice 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
- 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. 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 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. These coverslips are placed usually just on the fume adsorber for the coverslipping station. **Please use the long coverslips (e.g. 24x50 mm or 24 x 60 mm) only if you have those many sections on a slide. Alternatively use smaller size coverslips**
- **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 (in the IHC work area, opposite the microwave)

H & E Staining Protocol for Cryo sections:

If tissue is not previously fixed, you need fixation step first. If already fixed, ignore the 4%PFA Step. Alternatively fix in ice cold acetone. Talk to Shreyas if you have queries

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|-----|------------------|---|
| 1 | 1H – overnight | Thaw frozen slides at room temperature |
| 2. | 5 minutes: | 4% PFA (Only if tissue is not previously fixed). |
| 3. | 3 minutes | Rinse in 1X PBS-1 |
| 4. | 3 minutes | Rinse in 1X PBS-2 |
| 5. | Rinse: | Distilled Water – BLUE BUCKET |
| 6. | 0.5 - 3 minutes: | Harris Hematoxylin (varies with type and thickness of tissue) |
| 7. | Wash: | Tap Water (2-3 changes) – WHITE BUCKET |
| 8. | 2 dips: | Acid Alcohol (0.5% HCl in 95% Ethanol) |
| 9. | Wash: | Tap Water (2-3 changes) – WHITE BUCKET |
| 10. | 5 seconds: | Saturated Aqueous Lithium Carbonate |
| 11. | 3 minutes: | Running Tap Water – WHITE BUCKET |
| 12. | Rinse: | Distilled Water – BLUE BUCKET |
| 13. | 1 minute: | Eosin |
| 14. | 1 minute | 70 % Ethanol |
| 15. | 1 minute: | 95 % Ethanol |
| 16. | 1 minute: | Absolute Alcohol – 1 |
| 17. | 1 minute: | Absolute Alcohol – 2 |
| 18. | 1 minute: | Absolute Alcohol / Xylene |
| 19. | 1 minute: | Xylene – 1 |
| 20. | 1 minute: | Xylene – 2 |
| 21. | 1 minute: | Xylene – 3 |
| 22. | 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 Tissues Version 2.0 September 2021 by Adi Manek and H Neufeld
2. June 2022- Updated for facility by Shreyas Jois and Adi Manek with facility specifics

Other references:

1. <https://www.novusbio.com/support/fixation-and-permeabilization-in-icc-if>
2. <http://www.histosearch.com/histonet/Oct02A/fixationoffrozensections.html>