



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 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
- 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

- 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

Alcian Blue and Alizarin 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 (Varies with type and thickness of tissue)
10. Wash: Tap Water (2-3 changes) – WHITE BUCKET
11. 2 minutes: Running Tap Water – WHITE BUCKET
12. Rinse: Distilled Water – BLUE BUCKET
13. 30 s – 5 minutes: Alizarin Red Solution (Usually 2 minutes will produce nice red - orange staining of calcium.)
14. Shake off excess dye and blot sections
15. 20 dips: Acetone
16. 20 dips: Acetone-Xylene (1:1)
17. 1 minute: Xylene – 1
18. 1 minute: Xylene – 2
19. 1 minute: Xylene – 3
20. 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!!!!**

Results:

Strongly acidic sulfated mucosubstances, (cartilage) ----- blue

Calcium deposits (except oxalate) ----- orange-red

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.

Alizarin Red Solution:

Alizarin Red S (C.I. 58005) ----- 2 g

Distilled water ----- 100 ml

Mix well. Adjust the pH to 4.1~4.3 with 10% ammonium hydroxide. The pH is critical, so make fresh or check pH if the solution is more than one month old.

Acetone (100%)Acetone-Xylene:

Acetone (100%) ----- 50 ml

Xylene ----- 50 ml

Revisions:

July 2022- The protocol was updated from the references below for the histology core facility by Shreyas Jois and Adi Manek with facility specifics

References:

1. http://www.ihcworld.com/protocols/special_stains/alcian_blue.htm
2. http://www.ihcworld.com/protocols/special_stains/alizarin_red_s.htm
3. <https://www.statlab.com/pdfs/ifu/stare.pdf>