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

Oil Red O Staining of cryo-sectioned 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 scale. 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
- **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
- **For Oil Red O staining, use glycerine jelly or other aqueous mounting medium, ask Shreyas if unsure.**
- 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**
- 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)

Oil Red O Staining Protocol for Cryo sections:

If tissue is not previously fixed, you need fixation step first. If already fixed, ignore the fixation Step. Talk to Shreyas if you have queries

1	1H – overnight	Thaw frozen slides at room temperature
2.	2-10 minutes	4% PFA or 10%NBF (Only if tissue is not previously fixed).
3.	1 minute	Immerse in tap water (two changes) WHITE BUCKET
4.	2 minutes	60% isopropanol, to avoid carrying water (go to next step without rinsing)
6.	15 minutes	**Oil Red O (freshly prepared working solution)**
7.	Rinse	60% isopropanol (for tissue differentiation)
8.	1 minute	Immerse in distilled water (twice) BLUE BUCKET
9.	**15 seconds**	**Harris Hematoxylin**
10.	1 minute	Immerse in tap water (two changes) WHITE BUCKET
11.	1 minute	Immerse in distilled water (two changes) BLUE BUCKET

Leave slides in **water** until ready to coverslip; **don't let them dry out!**

Coverslip slides in **glycerine jelly** or another **aqueous mounting medium** in the Coverslipping station.

Results

- lipid.....red
- nuclei.....blue

Notes

1. Include a positive control- liver, negative control- paraffin processed lung
2. The section must not be taken through ethanol and clearing solvent prior to mounting, as this will remove the lipids.

Reagents

1. 0.5% Oil Red O Solution:

   Oil Red O (CI 26125) ---------- 0.5 g
   Isopropanol, 100% ---------- 100 ml

Dissolve the dye in the isopropanol, using the very gentle heat of a water bath. This is the stock stain. CARE - fire hazard

2. Oil Red O working solution
For use: Dilute 30 ml of the stock stain with 20 ml of distilled water, allow to stand for 10 mins, and filter into a Coplin jar, and cover immediately. The stain does not keep and should be made up fresh from the stock solution each time.

3. Aqueous Mounting Medium (glycerol gelatin)

	Kisser's	Kaiser's	Glycerol jelly	Modified
Gelatine	10 g	40 g	65 g	5 g
Distilled water	35 ml	210 ml	300 ml	50 ml
Glycerol (glycerin)	30 ml	250 ml	100 ml	50 ml
Phenol (carbolic acid)		5 ml	5 ml	

Mix gelatin and distilled water. Heat in 50 C oven until gelatin is dissolved (it takes about 30 minutes). Add glycerin and adjust pH to 7.0 using 1N NaOH (to avoid fading of hematoxylin). Store at room temperature. Warm to 37-40 C before mounting. Note: Modified protocol works well.

Revisions:

1. Based on H&E STAINING PROTOCOL-FROZEN SECTIONS Version 1.0 June 2022 by Shreyas Jois and Adi Manek
2. January 2023- Updated for facility by Shreyas Jois and Adi Manek with facility specifics

Other references:

1. **http://www.ihcworld.com/_protocols/special_stains/oil_red_o.htm**
2. **http://www.ihcworld.com/_protocols/special_stains/oil_red_o_ellis.htm**
3. **https://pharm.ucsf.edu/xinchen/protocols/oil-red-o**
4. **https://www.sigmaaldrich.com/deepweb/assets/sigmaaldrich/product/documents/259/600/mak194bul.pdf**
5. **https://www.statlab.com/pdfs/ifu/KTORO.pdf**
6. **http://www.ihcworld.com/_protocols/histology/mounting_medium.htm**