EHT embedding for cryostat

2025-06-15T18:58:45+01:00

protocol

DAY 01

1. Fixation 4% PFA for 30 minutes
2. Wash with PBS
3. Incubate with 15% sucrose and 0.03% Eosin from the stock overnight until they sink at 4C

DAY 02 prerequisites

- pre-freeze isopentane at -80C (it is normally stored at 4C due to its low boiling point)

Good to know before starting

- isopentane is being re-used - when finished embedding, collect used isopentane to designated bottle, try to prevent crystals from getting to the collection bottle
- isopentane is toxic, hence contact with sewage water is forbidden - only chemical waste can be used for disposal
- there are three sizes of molds (small, medium, big) - for EHTs we used the middle ones, but the small ones might also be usable based on the sample size. The big ones are too big for EHTs, these are used e.g. for spinal cord samples.

DAY 02

4. Prepare dry ice and isopentane
   -> How?

   Put dry ice to polystyrene container, then take metal container (which is stored in the closet in histology) and pour cold isopentane to the metal container. Put the metal container with isopentane inside the polystyrene container. Be careful to put proper volume of isopentane - too little would cause difficulties with freezing, too much would lead to isopentane pouring inside the mold and would cause freezing failure which would result in sample damage. The sufficient volume of isopentane is just to cover the whole bottom of the mold without pouring inside. Since the isopentane vaporizes, check also during samples processing that you still have enough of it and if not refill.

5. Prepare the working place: underlying foil, tweezers, OCT (labelled MBD), paper towels, samples

6. Put small amount of OCT at the bottom of the mold you will use. Make sure there are no major bubbles in OCT around the central area where you will be putting the sample.

7. EHT is stored in Eppendorf tube, sometimes it is difficult to get it out
   -> What worked the best for us?

   Prepare the underlying foil. Make sure the EHT is not stuck to the tube wall. Once EHT is floating, try to put the tube bottom upwards quickly without touching the desk. If the EHT still remains inside the tube, collect the poured liquid from foil using pipette and repeat the process until you succeed.

8. Grab the EHT from the underlying foil carefully using tweezers. Do not fully press the tweezers since you would destroy the tissue. Carefully transfer to the mold. Try to put to the central position. Once set, you can gently adjust positioning using tweezers.
9. Put the mold with sample to the container with isopentane and immediately add OCT (remember to always keep the OCT bottle bottom upwards to prevent bubbles in the liquid).

   Why we add OCT as quickly as possible?
   If added too late, crystals will form in the tissue; if added before putting to isopentane, based on our sample size, the sample might float away from our position.
10. Leave the sample in isopentane until OCT fully solidifies (white color) and then remove from isopentane using tweezers (not to burn yourself), wrap it in aluminium foil (+ label it) and put it to closed box with dry ice.

Proceed with other samples, once have all samples embedded, put all of them inside labelled paper box and in box with dry ice (CLOSED) transfer to the freezer room as quickly as possible.

Store at -80C.

When finished

- Disinfect the whole working area using GMO disinfection (big white bottle).
- Trash used tips to the waste bottle.
- Used liquids are put to the chemical waste, isopentane is put to the designated bottle for re-usage.
- Wash tweezers and splash them with disinfection.
- Put all the things back to designated places.
- Keep the big polystyrene box in histology, do not leave it in freezer room.

Q&A

- EHT positioning: Orientation of EHT within the mold does not matter - you will position during cryostat sectioning presets.
- Interval for cryostat sectioning: By default done the next day. However, after few hours in freezer should be possible to do cryostat sectioning within the same day as embedding. It is not recommended to continue sectioning immediately after embedding since only after the isopentane incubation the sample might not be fully frozen.