

1.1. Collection and preservation of heart tissue for histological and biochemical studies

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A. OBJECTIVE

This procedure can be used to dissect mouse heart samples for preparation of unfixed frozen cryosections or for isolation of RNA, DNA or protein.

B. CAUTIONS

- **Avoid at all costs submerging any of your body parts in liquid nitrogen (LN₂) since it is a cryogen that is extremely harmful to live tissue.**
- Freezing tissues for isolation of RNA, DNA or protein or preparation of unfixed frozen tissues should be carried out as quickly as possible after euthanasia of the animal to maximize the integrity of the sample. Therefore, speed is more important than the careful surgical procedures that are required for surgery on living animals.
- All handling of live animals must be carried out only by individuals who are listed as authorized personnel on an approved Institutional Animal Care and Use Committee (IACUC) protocol. Animals must be euthanized only according to the procedures approved in an approved protocol.

C. MATERIALS

- Dissection tools: scissors and forceps
- Microcentrifuge tubes
- Long forceps for freezing
- Aluminum foil cups, cork discs, or other mount for histological sample freezing
- Isopentane (aka 2-methyl-butane)
- Liquid nitrogen in Styrofoam container
- OCT (Optimal Cutting Temperature media)
- Ethanol squirt bottle
- Styrofoam boards and pins
- Baggies for tubes of tissues in freezer
- Parafilm or saran wrap

D. METHODS

PREPARATION

1. A beaker 3/4 filled with isopentane (2-methyl butane) should be placed in a Styrofoam container, which is filled with liquid nitrogen just lower than the level of isopentane in the beaker.

Alternative: Dry ice can be used to cool isopentane and may result in less cracking of tissue than isopentane over liquid nitrogen.

2. Make aluminum foil cylindrical containers with a diameter of about 1 cm and no larger than 1 cm high using an appropriate mold **{do you have a picture of the one you are using?}** and

extra-heavy duty aluminum foil (Fig. 1). Label the cups using the mold for support with the mouse/sample number and the tissue (In the example in Figure 1, “H” is used to indicate it is heart tissue) at the bottom of the cylinder and 2 times on the sides (1 per tissue) with an extra-fine Sharpie marker. Prepare 1 for each tissue to be used for preparation of unfixed frozen histological sections. Label microcentrifuge tubes or cryovials for each tissue to be frozen for RNA , DNA or protein isolation.

Alternative: Cork discs, slices of dowel rods, or other containers that will not crack in liquid nitrogen can be used to support the tissue for histological sections instead of foil cups.



Fig. 1. Making and labeling a foil cup.

3. While the isopentane is cooling, set up your dissection area and tools. Place Saran Wrap or parafilm to cover surface of Styrofoam board for dissection area. Ensure that dissection tools are clean and wiped off with ethanol.

PROCEDURE

1. Pin hands and feet of an euthanized mouse spread apart, ventral (belly) side up, on Styrofoam board.
2. Spray enough 95% ethanol on ventral surface of mouse to wet down fur, and pat dry with Kimwipes.
3. Pick up forceps in your non-dominant hand and strongly grab skin of mouse below the sternum and pull up toward you.
4. Pick up scissors in your dominant hand and make as deep an incision as possible under the forceps by pointing the scissor tips perpendicular to the Styrofoam board. A proper incision will cut through all layers of skin and the peritoneum so that you can visualize internal organs.
5. Reposition forceps on the anterior flap of the primary incision and make a secondary incision anterior and perpendicular to the primary incision up the midline of the thoracic cavity.
6. Make additional lateral incisions on the right and left side of the secondary incision parallel to the primary incision so that the skin of the thoracic cavity can be pinned out on the Styrofoam board.
7. Using curved blunt forceps, carefully lift the heart out of the thoracic cavity and cut through the vessels connecting to the heart to remove the heart from the body.

Alternative: If the heart is to be used for histology, the heart can be perfused with 1M KCl by direct injection (~200 μ l) into the right atrium until the heart ceases to beat (this ensures that the heart is stopped in diastole and allows better comparison of transverse histological sections).

8. The heart is then rinsed in PBS and gently squeezed with blunt forceps to eject blood from the chambers.

Note: If mass is required, the heart is dabbed dry and quickly weighed.

9. If the whole heart will be used for preparation of unfixed frozen histological sections, the heart can either be covered in OCT and frozen APEX up or cut into 2-3 transverse pieces using new ethanol-cleaned razor blades. For mounting in foil cups, first place enough OCT in the foil container to just cover the bottom.

Alternative: If the heart will be used for biochemical studies it can be placed in a labeled microcentrifuge tube or cryovial, snap frozen by submerging in liquid nitrogen, and stored at -80°C. If both biochemical studies and histology from the same heart are preferred, the heart can be cut in half transversely using new ethanol-cleaned razor blades. The top half (containing atria) can be used (cut side down in foil cup or facing up on disc) to cut cryosections from the ventricles. The bottom half can be snap frozen to produce ventricle-only protein or RNA.

10. Add the tissue pieces to the cup, cut size down. Push the tissue down to bottom of cup. Add more OCT if tissue is not completely submerged. Add only enough OCT so tissue is just submerged.

Note: If there are any breaks in the light reflecting off the top of the OCT, then the tissue is not completely submerged and freezing damage will occur. If too much OCT is added, the tissue will take too long to freeze and the histology will not be high quality. It is important that just enough OCT is used to completely cover the tissue.

11. Carefully pick up the foil cup (or disc) from the edges with long forceps. Hold foil cup and place on the surface of the isopentane frozen in liquid nitrogen (**Fig. 2**). DO NOT IMMERSIVE IN ISOPENTANE! You want a slow freeze. Proper freezing should proceed from the outside in, with the circle of unfrozen OCT getting smaller and smaller until it disappears and the entire sample is frozen.

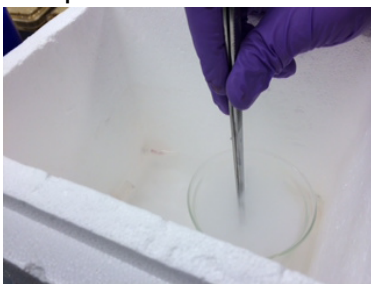


Fig. 2. Freezing sample for histology in a beaker of isopentane in liquid nitrogen.

12. When the OCT is completely frozen, place the foil container in the liquid nitrogen, until you have collected all samples and are ready to store them.

13. Mounted hearts can be stored for long periods of time at -80°C *WITHOUT ANY THAWING* prior to cryosectioning. 8 µm sections should be cut from the bottom of the sample (facing the bottom of the foil cup) onto Superfrost Plus (positively-charged) microscope slides.

E. EVALUATION AND INTERPRETATION OF RESULTS

- This procedure does not constitute an experiment in itself. However, before proceeding with downstream histological experiments, the quality of the sections should first be verified by routinely staining 1 slide cut from each sample with a general histological stain such as hematoxylin & eosin to ensure section quality before proceeding with “Immunofluorescence of Unfixed Cryosections”.
- Controls to verify the quality of RNA or protein isolation from samples frozen for biochemical analysis should also always be used before interpreting the results of downstream experiments.

F. REFERENCES

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