

## MOUSE ORGAN HARVEST PROTOCOL 10/01

### TO FREEZE TISSUES FOR FROZEN SECTIONS:

- 1. Make 1:1 OCT: PBS for tracheal infiltration to inflate lungs before freezing**
2. Get bench area ready with absorbent material, styrofoam board and dissection equipment
3. **In a small styrofoam flat container, make a slurry with small bits of dry ice and 2 methyl butane. This will be the freezing mixture which will freeze the organs in OCT for frozen sections**
4. Lay euthanized animal on dissecting board
5. IF necessary, Perfuse animal with PBS and then with freshly made 4% paraformaldehyde
6. Expose trachea and infiltrate lungs with OCT/PBS mixture. Remove entire block and place on paper towel to drain before freezing flat in vinyl mold with OCT.
7. Harvest tissues and place in sequence onto paper towels to dry off tissue fluids before placing into vinyl molds, flat on the bottom and then fill with OCT. This keeps the tissues all at the same level:
  - A. spleen/thymus, lymph nodes. adrenal**
  - B. Liver, pancreas, kidney**
  - C. Heart, OCT inflated and drained lungs**
  - D. GI (small intestine, colon, ? stomach) and GU (Bladder, prostate, testis, ovary, uterus)**
  - E. Skin, skeletal muscle, salivary glands, mammary glands, (decalcified femur for blocks)**
  - F. Brain**

IT IS BETTER TO KEEP THESE AS SHOWN. CAN COMBINE A,B,C and D,E,F WHEN CUTTING FROZEN SECTIONS SO THAT YOU WILL GET 2 SLIDES PER ANIMAL
8. **Place plastic molds containing organs and OCT into dry ice/ isopentane slurry till OCT turns white**
9. Remove plastic molds with frozen organs from dry ice/ 2 methyl butane and let them sit for a minute or so on paper towels, snap off the plastic molds and place frozen blocks into labeled bags for storage in labeled boxes in the second container of dry ice for storage at minus 70.

### FOR PROCESSING INTO PARAFFIN BLOCKS

1. Use labeled plastic cassettes to place organs in above order, snap shut and immerse into 10% buffered formalin.
2. Let the organs fix for at least 24 hours (may go longer)
3. Move fixed organs into 70% alcohol after 24 hours if needed for immunohistochemistry.
4. Deliver to Core lab for processing into paraffin blocks

### SUPPLIES:

1. OCT Compound: VWR Cat No: 25608-930
2. Vinyl molds: VWR Cat No 15160-215
3. Frozen sample write-on bags: VWR Cat. No: 01-002-37
4. 2 methyl butane: Fisher Cat. No. 03551-4

