

Lung Isolation—For Histological Analysis

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BEFORE BEGINNING SURGERY, you will need:

- Avertin (1X stock in 4°C under bench)
- Forceps, surgical scissors, pins, dissecting platform
- Cold PBS (if perfusing)
- 10, 3, and 1 mL syringes
- 25G needles
- 10% Formalin (RT)
- 50 mL conical tubes

- 1) Anesthetize mouse with avertin, test with forceps (toe pinch) to make sure under
 - a. Can use a lethal dose if not perfusing
- 2) Spray down mouse with 70% ethanol
- 3) Make initial incision just below the rib cage. Cut through skin and connective tissue across the mouse. Make lateral incisions on each side up to the neck of the mouse.
- 4) Quickly cut into ribcage
 - a. **If perfusion is required:** perfuse 10 mL of cold PBS (use a butterfly needle) through right ventricle until lungs cleared of blood (cut a slit in left ventricle to allow blood to leave)
 - i. Cut out heart to euthanize mouse (can use Kimwipe to soak up blood)
- 5) If saving any part of the lung (not to be fixed) remove it now. If this section of the lung needs to be inflated with another solution, this can be done in a Petri dish.
- 6) Pull skin back above the head of the mouse to expose the throat area
- 7) Carefully remove the rest of the rib cage (the more bone removed the better) and other tissue to expose trachea, place forceps under trachea to keep exposed (making sure to separate the trachea from the esophagus)
- 8) Inject 10% Formalin into trachea (go between the cartilage rings) until the lungs inflate—you should be able to do this with 2-3 mL
- 9) Dissect out the lungs by gently tugging on the trachea while snipping away the connective tissue; leave lungs intact
- 10) Drop the entire set of lungs into a 50 mL conical with enough 10% Formalin to cover all of the tissue (about 3X volume of tissue) and let sit overnight
- 11) **The following day:** Trim other tissues off lung, dissecting off each lobe. Drop the lobes into a histology cassette and store in 70% EtOH