# LUNG EXCISION AND SLICING PROTOCOL

TECHNOTE 022
NOVEMBER 11TH, 2024

## PURPOSE

The purpose of this document is to provide guidance through the lung excision and slicing process to obtain murine Precision Cut Lung Slices (PCLS).

## LEGAL DISCLAIMER



## TECHNICAL SUPPORT CONTACT INFORMATION

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# 1. REAGENT PREPARATION

## 1.1 1XHBSS/HEPES SOLUTION (2L)

### 1.1.1 REAGENTS

- 200mL of 10xHBSS (Life Technologies, Cat# 14065-056)
- 4.76g of HEPES (Life Technologies, Cat# 15630-080)
- >1800mL of sterile DI water
- Sodium Hydroxide solution

### 1.1.2 MATERIAL

- 1 4L Erlenmeyer
- 1 magnetic stirring bar
- Pipette tips
- Weigh boat

### 1.1.3 EQUIPEMENT

- pH-meter
- Magnetic stirring plate
- 1000uL pipette
- Scale (0.01g)

### 1.1.4 INSTRUCTIONS

1. Using the calibration solutions, calibrate the pH sensor, as per manufacturer's instructions.
2. Place the 4L Erlenmeyer on the magnetic stirring plate and insert the magnetic stirring bar.
3. Add 200mL of 10xHBSS in the 4L Erlenmeyer.
4. Power on the stirring plate to a medium intensity stir.
5. Add approximately 800mL of sterile water to create a 1L solution in the Erlenmeyer
6. Add 4.76g of HEPES to the solution.
7. Once all HEPES is dissolved, measure the pH of the solution using the calibrated pH-meter.
8. Using the 1000uL pipette, add 10M Sodium Hydroxide (<3mL) until the pH of the solution reaches 7.4.
9. Add the remaining 1200mL of sterile water and let stir for a few minutes to ensure homogeneity.

## 1.2 2% AGAROSE (50ML)

### 1.2.1 REAGENTS

- 1 g of Agarose (Invitrogen, Cat# 16520-050) kept desiccated at room temperature.
- 50mL of 1xHBSS/HEPES solution

### 1.2.2 MATERIAL

- 1 100mL Erlenmeyer
- 1 100mL beaker
- 1 magnetic stirring bar

### 1.2.3 EQUIPMENT

- 1 Magnetic stirring heating plate

### 1.2.4 INSTRUCTIONS

1. Place the 100mL Erlenmeyer on the magnetic stirring heating plate and insert the magnetic stirring bar.
2. Add 50mL of 1xHBSS/HEPES solution in the 100mL Erlenmeyer.
3. Power on the plate to a high heat and medium intensity stir.
4. Once the HBSS starts boiling, gradually add the agarose powder into the stirring solution. Avoid integrating too quickly to prevent clumps.
5. Cover the opening of the Erlenmeyer with a Kim wipe to prevent evaporative losses.
6. Let stir for approximately 5 minutes at a very low boil.

# 2. LUNG EXCISION

## 2.1 REAGENTS

- Sodium pentothal, or preferred agent for euthanization
- Rubbing alcohol
- 3 bottles of 100mL of 1xHBSS/HEPES solution
- 30mL of 2% Agarose

## 2.2 MATERIALS

- 1 Straight forceps
- 1 Straight tweezers
- Curved scissors
- Straight scissors
- 4-0 USP surgical sutures
- 3 20G BD Saf-T-Intima™ IV Catheter
- 3 5mL syringes
- 1 1mL syringe
- 3 1mL syringe with needle
- Gauze
- Medical tape
- Ice bucket

## 2.3 INSTRUCTIONS

### 2.3.1 PREPARATION

1. **Critical step.** Prior to performing any experiments, obtain the appropriate approvals (e.g. IACUC) and trainings (e.g. animal handling, injections and surgical procedures).
2. Prepare a water bath and heat to 44C.
3. Microwave a 400mL beaker of water for 2 minutes to make the water boil.
4. Place 2 vials of agarose in the beaker of boiling water for up to 5 minutes.
5. Once the agarose is melted, place the agarose tubes in the water bath to cool them to 44C.
6. Gather the autoclaved forceps and scissors.
7. Place the 3 HBSS bottles in a Styrofoam box and fill with ice.

### 2.3.2 EUTHANASIA

1. Weigh the mouse using a scale. **!**Note down the mouse's weight.
2. Euthanize the mouse using a lethal dose of sodium pentothal as specified in the protocol approved by the institution's animal care and use committee.
3. Test the mouse's reflexes by squeezing its paw to verify that it is sufficiently anesthetized.

### 2.3.3 DISSECTION

1. Tape the mouse's upper and lower limbs to extend its abdomen as much as possible.
2. Wet the mouse's fur by applying rubbing alcohol over its neck and abdomen.
3. Using the forceps, pull on the abdominal skin and use the curved scissors to make an incision from the lower abdomen to the clavicle.

Figure 1: Initial incision

4. Using the straight scissors, separate the skin from the neck's connective tissue by blunt dissection.
5. Once the skin is separated, pull it with the tweezers and cut from the sternum to the mouse's chin.
6. Using your fingers, pull on the skin on each side of the abdomen, thorax, and neck to separate the tissues and to remove the skin and expose the ribcage.
7. Using the forceps, pull on the abdominal muscles and use the curved scissors to cut from the bottom of the abdomen to the rib cage, exposing the viscera and diaphragm.
8. **Critical step.** Use the forceps to lift the rib cage and gently puncture the diaphragm with the scissors, being careful not to damage the lungs.

9. Using the curved scissors, expand to diaphragm opening. Remove the rib cage using the forceps and curved scissors, being extremely careful not to pierce or cut the lungs.

Figure 2: Removal of the rib cage

10. Using two pairs of forceps, separate the salivary glands and the neck's connective tissue to expose the trachea, which is recognizable due to its white ringed appearance.
11. Slide the closed straight scissors between the trachea and the oesophagus to separate both by blunt dissection.
12. Once the oesophagus is cleared, cut it with the straight scissors.

### 2.3.4 INTUBATION

1. Pass two sutures under the trachea using forceps.

Figure 2: Measurement of the trachea hole distance using the catheter

2. Poke a hole as far up in the trachea as possible and remove the needle from the catheter.
3. Insert the catheter in the hole.
4. Using the forceps and tweezers, tie two knots with the surgical twine to tighten the trachea on the catheter.

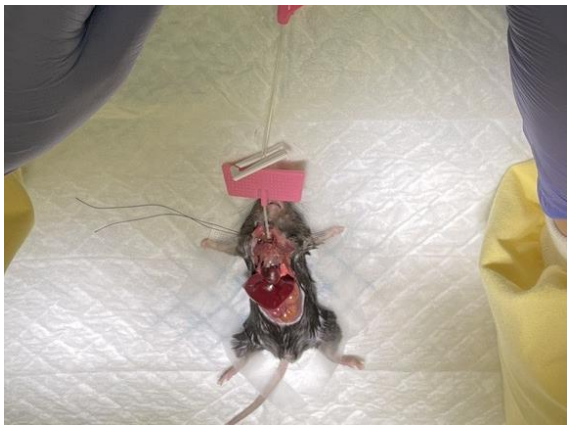

Figure 3: View of the intubated mouse

### 2.3.5 PERFUSION

1. Using a needle, poke a hole in the soft part of the catheter tubing, above the wings.

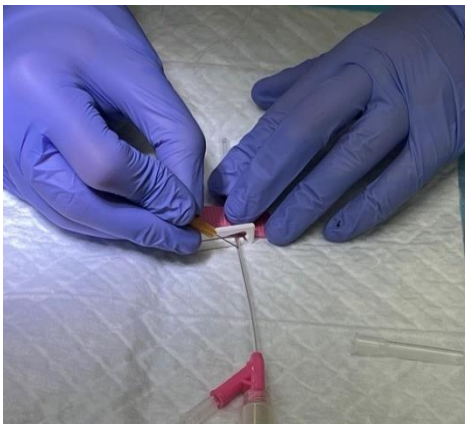

Figure 4: Hole location on catheter tubing

2. Take a vial of agarose from the water bath and pull 3mL of liquid agarose in a 5mL syringe.
3. Connect the agarose syringe to the catheter. Be careful not to pull on the catheter while doing so, which would remove it form the trachea.
4. Fill a 1mL syringe with air and connect to the other section of the catheter.
5. Squeeze the tube shut with the clamp between the lungs and the hole.
6. Push on the agarose syringe to fill the tube with agarose and for the air to exit through the hole. Stop when agarose reaches the hole.

7. Unclip the clamp and proceed to inject agarose into the lungs. Stop when the lingula of the left lung gets filled and erected.

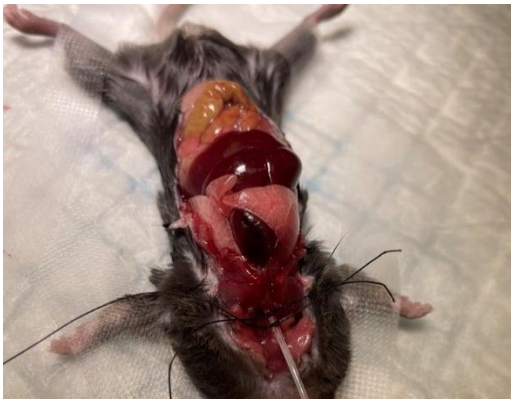

Figure 5: View of the lingula

8. Finish the injection by pushing 0.3mL of air after the agarose.
9. Close the clamp between the wings and the hole.
10. !Note the volume of agarose injected.
11. Cut the catheter above the clamp and throw away with the agarose syringe.

### 2.3.6 SOLIDIFICATION

1. Cover the mouse with a gauze and apply ice over and on the sides of the animal. Make sure to avoid direct contact of ice on the organs. !Note the time at which this step is done.
2. Wrap the mouse with the dissection sheet and put in a refrigerator to solidify the agarose.

### 2.3.7 REMOVAL OF LUNGS

1. Take out the mouse from the refrigerator after 20 minutes.
2. Remove the ice and gauze from the mouse.
3. Using the forceps and scissors, detach the lungs and heart from the thorax.
4. Place the lungs and heart in a 100mL bottle of HBSS on ice.

# 3. LUNG SLICING

## 3.1 REAGENTS

- Mouse lungs in 100mL of HBSS
- 2% agarose
- Super glue
- 500mL bottle of 1xHBSS
- 500mL bottle of sterile DMEM
- 5 vials of antibiotics

## 3.2 MATERIALS

- Scalpel
- Scalpel Blades
- Razor blade
- 24-well plate
- Tweezers
- 5mL syringe
- Dissection pad
- 1000uL pipette
- 1000uL pipette tips
- 100mL beaker

## 3.3 EQUIPEMENT

- Compresstome® VF-310-0Z (including):
    - Chilling block
    - Specimen tube

## 3.4 INSTRUCTIONS

### 3.4.1 PREPARATION

1. Add 5mL of 100x Antibiotic-Antimycotic to a 500mL bottle of DMEM. Mix well.
2. Place the bottle of DMEM in an incubator at 37C.
3. Place the chilling block in a -30C refrigerator.
4. Prepare a water bath and heat to 44C.
5. Microwave a 400mL beaker of water for 2 minutes to make the water boil.
6. Place 2 vials of agarose in the beaker of boiling water for up to 5 minutes.
7. Once the agarose is melted, place the agarose tubes in the water bath to cool them to 44C.
8. Pipette 1mL of DMEM supplemented with Antibiotic-Antimycotic into each well of a 24-well plate. To avoid contaminating the 500mL solution, decant required media to a sterile container first.
9. Cover the plate and put in the incubator at 37C with 5% CO2.

### 3.4.2 SPECIMEN TUBE PREPARATION

1. Remove the lungs from the bottle of HBSS and place them on the dissection pad.
2. Using a scalpel, carefully cut the left lung away from the right lung and the heart. Make sure not to damage the lung with the blade.

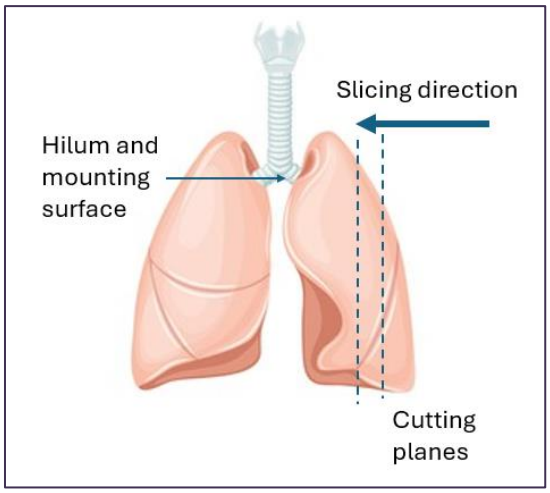


Figure 6: Lung slicing diagram

3. Fill the 5mL syringe with liquid agarose from the agarose tube.
4. Remove the chilling block from the refrigerator.
5. Apply a drop of super glue on the flat part of the specimen tube.
6. Glue the lung on its proximal edge on the specimen tube. If the lung is too long to fit in the specimen tube, trim a millimetre off the upper or lower lobes.

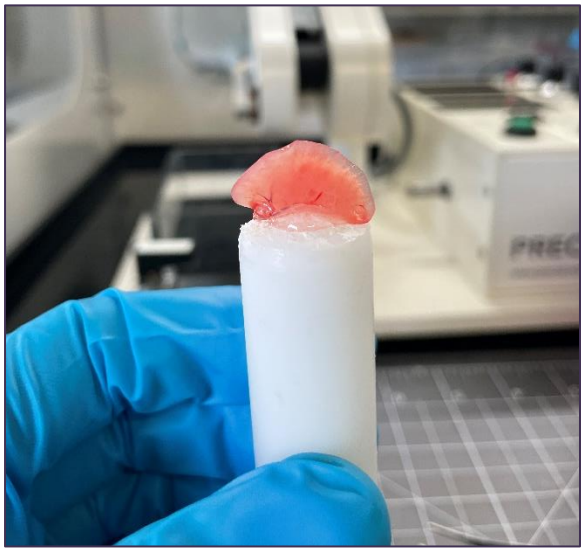

Figure 7: View of cut lung lobe on specimen tube.

7. Lift the metal part of the specimen tube to match the height of the lung's distal edge and fill the specimen tube with liquid agarose.

8. Place the specimen tube inside the chilling block and let it solidify for 2 minutes. Place the chilling block back in the freezer when finished.

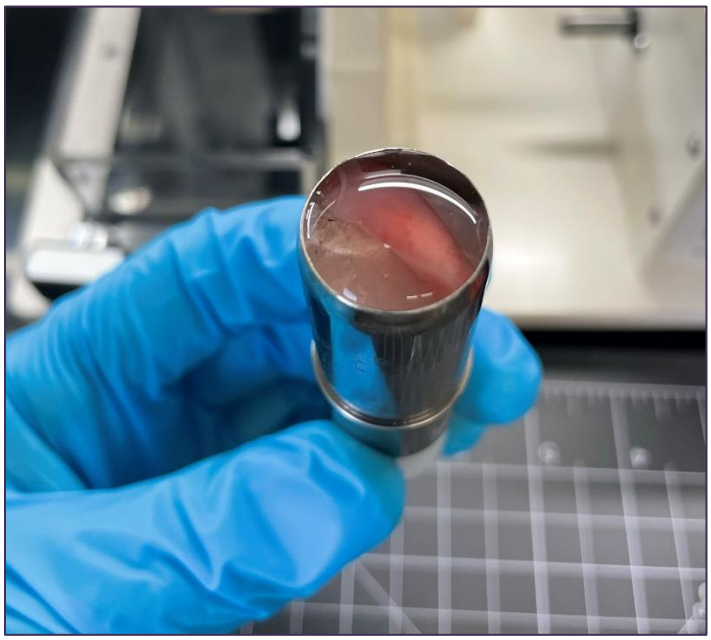

Figure 7: View of solidification setup.

### 3.4.3 COMPRESSTOME AND SLICING

Figure 8: Precisionary Compresstome

1. Insert the specimen tube in the buffer tray and secure into the Compresstome® using the screw knob.
2. Fill the buffer tray with 1xHBSS until it completely covers the specimen tube.
3. Add a razor blade on the blade holder.
4. Slide the blade holder on the Compresstome® and lock in place using the set screw and the designated Allen key. The round magnets should be facing outwards.

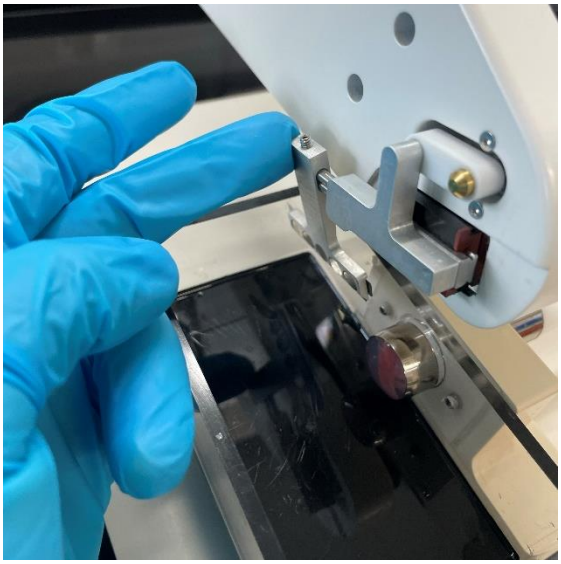

Figure 9: Adding the razor blade to the Compresstome with the tissue sample mounted.

5. Power on the Compresstome® using the switch on the side. Set the slice thickness to 250um, speed to 3 and the oscillation to 5 using the controller knobs.
6. Using the fast-forward switch, bring the plunger close to the specimen tube.
7. Remove the DMEM filled 24-well plate from the incubator.
8. Press the start button to begin slicing.
9. Using tweezers, take each lung slice and place them in separate wells in the 24-well plate.
10. Cover and label the 24-well plate and place in an incubator at 37C with 5% CO2.
11. Wash the slices twice, with fresh DMEM supplemented with antibiotics, before leaving them overnight, allow for at least an hour between the two washes. From the next day, wash daily.