



PRECISION-CUT LUNG SLICING

PROTOCOL FOR CUTTING SLICES OF FRESH LUNG TISSUE

Key to reading the protocol:

√ Rationale for procedural step

♠ Tips & Tricks

1. Anesthetize the animal, then dissect to cannulate the exposed trachea with a catheter.
2. Cool the [live lung specimen](#) with 4°C saline.
3. (Optional) Inject a bolus of agarose gel (2%) to fill the pulmonary artery.
 - √ This process of filling with agarose allow for inflation of alveoli with the same embedding material, and prevents the alveoli from collapsing during the cutting process. Some lung specimens may be sectioned without agarose infusion, especially if the lung specimens are from bovine or sheep animal models (because the quantity of agarose needed for infusion exceeds several liters).
4. Using a syringe, inject a bolus of air to clear the airway.
 - √ Injection of air helps infused agarose reach the alveoli.
5. Select a section of the lung lobe you would like to take tissues from for sections.
6. Glue the tissue sample onto the Compressstome® specimen syringe. Place the embedding cap onto the tube, then fill the syringe with 2% agarose (Sigma A-0701, low gelling point, incubated at ~37°C). Tap the side of the tube to dispel any bubbles from the agarose.
 - √ Loading the agarose with the embedding cap allows you to see if there are any bubbles surrounding the specimen *before* you draw the tube plunger down.
7. Draw the syringe downward to bring the lung tissue core sample into the syringe.
8. Cool the entire contents of the specimen syringe with the chilling block. The lung tissue sample is now embedded in agarose. The agarose will solidify enough for stable sectioning.

9. Load the specimen syringe onto the Compresstome® slicer.
10. The protocol is complete for preparing the lung tissue core specimen for sectioning.
Proceed from here with normal Compresstome® sectioning procedures.

♠ PCLSs produced can be maintained overnight in Dulbecco's modified Eagle medium (37°C, 5% CO₂) supplemented with 1% penicillin-streptomycin solution.

References

*** Uses the Compresstome® for successful lung tissue slices.**

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2. ***Hiorns JE, Bidan CM, Jensen OE, Gosens R, Kistemaker LE, Fredberg JJ, Butler JP, Krishnan R, Brook BS. Airway and Parenchymal Strains during Bronchoconstriction in the Precision Cut Lung Slice. Front Physiol. 2016 Jul 21;7:309.**
3. ***Oenema TA, Maarsingh H, Smit M, Groothuis GM, Meurs H, Gosens R. Bronchoconstriction Induces TGF- β Release and Airway Remodelling in Guinea Pig Lung Slices. PLoS One. 2013 Jun 26;8(6):e65580.**
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5. ***Yim PD, Gallos G, Perez-Zoghbi JF, Trice J, Zhang Y, Siviski M, Sonett J, Emala CW Sr. Chloride channel blockers promote relaxation of TEA-induced contraction in airway smooth muscle. J Smooth Muscle Res. 2013;49:112-24.**