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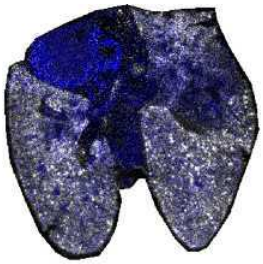
Mouse Lung Inflation Protocol for Mass Spectrometry Imaging (DMS22)



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We use this protocol and it's working

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Abstract

This protocol describes the preparation of inflated mouse lungs with gelatin for use in subsequent MSI analyses.

Guidelines

Gelatin (porcine) works well to preserve histology and ease serial sectioning. It is compatible with many downstream applications (mass spectrometry imaging of lipids, metabolites, glycans, and post-analysis immunohistochemistry, etc.). Compatibility with other downstream applications should be tested before use.

Troubleshooting

Before start

MATERIALS:

- Porcine gelatin, ~300g Bloom, Type A (Sigma #G1890)
- Molecular grade H_2O
- Microwave
- 50 mL conical tubes
- 200 mL glass Erlenmeyer flask
- 100-200 mL glass beaker filled with ~40°C water or hot metal beads
- 70% ethanol in a spray bottle
- Styrofoam board or other stable dissection surfaces
- Scissors
- Forceps or tweezers
- Surgical pins or metal needles (at least 6)
- Needle and suture (Ethicon Perma-Hand silk 3-0, BB 17mm 3/8 taperpoint, 30″ #K882H, non-cutting)
- Catheter needle with leur fitting (Jelco ProtectIV, Smiths Medical, #3066 pink 20G x 1 ¼″ radiopaque)
- 5 mL syringe with leur lock

PREPARATION:

- In a 200 mL erlenmyer flask, combine 100 mL molecular grade H2O and 2 g porcine gelatin.
- Microwave flask with high heat, frequently swilling to prevent the mixture from boiling over.
- Once the gelatin is completely dissolved, transfer to two 50 mL conical tubes.
- Place the tubes in a beaker filled with ~40°C water or hot metal beads and take to necropsy. The warm bath will prevent the spare tube of gelatin from cooling too much during the work.

PROTOCOL:

1 Euthanize mice according to IACUC approved protocols. Pinch the foot firmly using a thumb nail to ensure the mouse is dead and non-responsive.

2 Lay the animal on its back and pin its arms and legs to the Styrofoam board in an X-like shape. Pin the head back with one pin in the fore snout so the neck area is popped up to help expose the trachea.

3 Spray with 70% ethanol to keep fur down.

4 Open the skin along the midline, starting from below the abdomen and working up to the jaw. Open the skin and pin it down into the board.

5 Open the peritoneal cavity, pierce the diaphragm, cut along the anterior half of the rib cage on both sides and continue cutting up to expose the tracheal area. Be careful not to nick or cut the trachea itself!

6 Cut the collarbone (take care not to pierce major vasculature in area) to clear room for the lungs and trachea to be removed en bloc.

7 Remove the thymus (leave if desired).

8 Place two suture lines around mid-trachea and tie with loose butcher's knots. Leave them ready to pull.

9 Draw up ~2 mL of 2% gelatin in water (make sure it is NOT HOT! It should be between 25-37°C). Set aside within easy reach.

10 10. Insert the catheter needle into the trachea above the suture points, keeping the needle parallel to the trachea so it doesn't pierce through the top or bottom. Remove the metal needle and gently push the catheter to insert it farther.

11 11. Secure the catheter by tightening the sutures. Add a second knot if desired.

12 12. Attach the syringe to the secured catheter leur fitting.

13 13. Apply gentle pressure to fill lungs to capacity with gelatin – 1.5 +/- 0.25 mL. The lungs are filled when you see the accessory lobe fill to tip, with the other lobes filled.

14 14. When the lungs are full, firmly grasp catheter in the trachea at the point between the two sutures.

15 15. Remove the syringe by cutting the catheter tube above the grasp point.

16 16. Quickly scoop the whole inflated lungs and heart and heat the trachea above the sutures. Use scissors to relieve other attachment points (such as descending vasculature above the liver, etc.).

17 17. While still holding the trachea/catheter, place on a foil square and proceed to freezing. (Note: hold trachea/catheter until that area freezer to prevent backflow.)

18 18. Proceed to Tissue Freezing Protocol for MSI (DMS01).

Protocol references

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