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Dissection and immunohistochemistry of mouse lung

Seol-Hee Kim[1], Thomas Taylor-Clark[1]

[1]University of South Florida

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ABSTRACT

Mice are euthanized, perfused with fixative for lung tissue collection. Mouse lung is cryosectioned and slices are stained for protein expression using immunohistochemistry. Expression of specific proteins and reporter proteins are visualized using microscopy.

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Tissue collection

1 §6 to 8 weeks old mice (male) were euthanized by CO_2 inhalation.

2 §Mice were transcardially perfused with phosphate buffered saline (PBS) to remove blood followed by 3.7% formaldehyde (PFA).

3 §Larynx, Trachea and lung were collected.

4 §Tissue was post-fixed with 3.7% PFA for overnight at 4 °C with gentle agitation.

Cryosection

5 §Tissue was washed with ice-cold PBS three times for 30 min.

6 §Tissue was transferred to 30% sucrose solution for cryoprotection and incubated at 4 °C with gentle agitation until it sinks to the bottom of the tube.

7 §Lung was flushed 3 to 4 times with PBS containing mL syringe attached with a 23-guage blunt needle, followed by inflating the lung with 2% low melting agarose solution.

8 §After the agarose solution become solidified, the lung lobes were separated and mounted with OCT for cryoprotection prior to the snap frozen in dry ice.

9 §Tissue was cut at 80 µm thickness, and the slices were collected in cryoprotectant filled well-plates and stored in -20 °C for further use.

Immunohistochemistry

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10 §Tissue slices were washed with PBS three times for 10 min.

11 §Tissue slices were permeabilized with 0.3 % PBSTx (PBS with 0.3% triton x-100) for 20 min.

12 §Tissue slices were blocked with blocking buffer (1% bovine serum albumin/10% normal donkey serum/0.3% PBSTx) for 1.5 h at room temperature.

13 §Tissue slices were incubated with primary antibodies diluted in blocking buffer overnight at 4 °C.

14 §Tissue slices were washed with 0.2% tween20 in PBS (PBST) for 20 min for three times at room temperature.

15 §Secondary antibodies were diluted in the 1% bovine serum albumin/5% normal donkey serum/0.2% PBST and tissue were incubated with it for 2 h at room temperature.

16 §Tissue slices were washed with 0.2% tween20 in PBS (PBST) for 20 min for three times at room temperature.

17 §Rinse tissue slices with PBS three times to remove detergent and cover-slip with VECTASHIELD® Antifade Mounting Medium with DAPI.

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