

Immunofluorescence- NKX2.1, [EMCN](#), and [ACTA2](#)

Purpose: Immunofluorescence on slides of 7µm frozen sections of C57BL6 E16.5 lungs for NKX2.1, [EMCN](#), and [ACTA2](#) with antigen retrieval.

Day 1

1. For Frozen tissue, rinse 2X in PBS, then 5 min in 4% PFA/PBS, then rinse 1X in PBS.
2. Briefly equilibrate slides in Antigen Retrieval Buffer.
3. Antigen retrieval, pH 6.0 (times will vary according to microwave).
4. 10 mM sodium citrate, pH 6.0, and heat in a microwave at 96°C. *We usually do a series of three runs (6-7 minutes each run) to equal the time/temp because of evaporation (refill coplin jars with dH₂O).
5. Microwave according to instructions on microwave.
6. Cool on countertop, 15 min.
7. Rinse with dH₂O
8. 1X PBS, 5 min.
9. Block in 4% Donkey serum/PBS-T, 2 hours at RT.
10. For Rabbit NKX2.1 (R1231) dilute 1:1000. For goat anti-[EMCN](#) ([Endomucin](#), AF4666, R&D Systems) dilute 1:200, for mouse anti-[ACTA2](#) ([α Smooth muscle actin](#), A5228, Sigma) dilute 1:2000 in blocking buffer. Spin down in µfuge for 10 minutes and incubate on tissue overnight @ 4°C.

Day 2

1. Rinse slides in PBS-T 3X, 5 min.
2. Apply secondary antibody , Donkey Alexa Fluor 488 anti-rabbit IgG ([A21206](#), Lot# 1608521, for anti-NKX2.1), Donkey Alexa Fluor 568 anti-goat IgG ([A11057](#), Lot# 1485187, for anti- [EMCN](#)) at 1:200, Donkey Alexa Fluor 647 anti-mouse IgG ([A31571](#), Lot# 1549801, [ACTA2](#)) at 1:200, in blocking buffer. Spin down in µfuge for 10 min, apply to tissue and incubated at room temperature for 1 hour.
3. Rinse in PBS-T 3X, 5 min.
4. Dilute DAPI 1:2000 and apply to slides for 10 min.
5. Wash in PBS-T 3X, 5 min.
6. Rinse slides in 0.1M PB 3X for 5 min.
7. Add 1 drop of Prolong Gold anti-fade mounting medium (P36930).
8. Coverslip with Gold Seal Coverslip (Cat# 3422 Electron Microscopy Sciences, 22 X 22 mm).
9. Allow Prolong Gold to cure overnight at room temp in light sealed box.

Tissue Used: LMM.14.57.4.51
E16.5 C57BL6
Gender: Female
Crown-to-Rump= 16.0mm
Weight= 0.574g

Appendix

Antigen Retrieval Solution

18ml Solution A- 0.1M Citric Acid (Sigma, C1909) pH 2.5
82ml Reagent B- 0.1M Sodium Citrate (Sigma, S4641) pH 8.2
1L dH₂O
pH 6.0