

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

Purpose: Immunofluorescence on slides of 7µm frozen sections of C57BL6 PND28 lungs for [HOPX](#), NKX2.1, and [ACTA2](#) with antigen retrieval.

Day 1

1. Paraffin sections are placed at 60°C for 2 hours to overnight to melt paraffin.
2. Paraffin sections are then placed in xylene 3X for 10 minutes each, followed by 3X in 100% ETOH for 3 minutes each, 95% ETOH for 3 minutes each, and 70% ETOH for 3 minutes each. Slides are then placed in 1X PBS for 5 minutes to completely rehydrate tissue.
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% Goat serum/PBS-T, 2 hours at RT.
10. For Rabbit anti-[HOPX](#) ([SC-30216](#), Lot # C2212, Santa Cruz) dilute 1:200, For guinea pig anti-NKX2.1 (G237, Bl. 10/7/11) 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, Goat Alexa Fluor 488 anti-rabbit IgG ([A11034](#), Lot# 1298480, for anti-[HOPX](#)), Goat Alexa Fluor 555 anti-guinea pig IgG ([A21435](#), Lot# 1571699, anti-NKX2.1) at 1:200, Donkey Alexa Fluor 647 anti-mouse IgG ([A31571](#), Lot# 1549801, anti-[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.14.19A.5.14
PND28 C57BL6
Gender: Male
Weight= 13.6g

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