

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

Purpose: Immunofluorescence on slides of 5µm paraffin sections of C57BL6 E16.5 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. Briefly equilibrate slides in Antigen Retrieval Buffer.
4. Antigen retrieval, pH 6.0 (times will vary according to microwave).
5. 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).
6. Microwave according to instructions on microwave.
7. Cool on countertop, 15 min.
8. Rinse with dH₂O
9. 1X PBS, 5 min.
10. Block in 4% Goat serum/PBS-T, 2 hours at RT.
11. For Rabbit [HOPX](#) ([SC-30216](#), Lot # C2212, Santa Cruz) dilute 1:200, For guinea pig anti-NKX2.1 (G237, Bl. 10/7/11) dilute 1:500, 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# 1366514, [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.14.24.8.24
E16.5 C57BL6
Gender: Male
Crown-to-Rump= 16.0mm
Weight= 0.592g

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