

Immunofluorescence-PECAM1, NKX2.1, ACTA2

Purpose: Stain slides of 7µm frozen sections of postnatal human lung from HTC for **PECAM1**, **NKX2.1**, **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% Goat serum/PBS-T, 2 hours at RT.
10. For mouse anti-**PECAM1** (**BBA7**, R&D Systems) dilute 1:100, for rabbit anti-NKX2.1 (RB TTF-1 1231, Lot# 0912A) dilute 1:1000, for mouse anti-**ACTA2** (**α Smooth muscle actin**, A5228, Sigma) dilute 1:2000 in blocking buffer. Spin down in µfuge for 10 min, apply to tissue and incubated O/N @ 4°C.

Day 2

1. Rinse slides in PBS-T 3X, 5 min.
2. Apply secondary antibody , Goat Alexa Fluor 488 anti-mouse IgG1 (**A21121**, anti-**PECAM1**), Donkey Alexa Fluor 555 anti-rabbit IgG (**A21428**, anti-**NKX2.1**) at 1:200, Donkey Alexa Fluor 633 anti-mouse IgG2A **A21136**, Lot# 1345055, **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 or TB, 3X, 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.
10. Store slides in a light-sealed box at 4°C.

Tissue Used:

LMH-15-D003-LLL-4B2.8

Gender: Male

Age: 9 Months

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