

Purpose: Stain slides of 7µm frozen sections of PND03 NKX2.1, SCGB1A1, and **TUBA1A** 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. 10 mM sodium citrate, pH 6.0, and heat in a microwave for 15 min at 90°C. *We usually do a series of three runs to equal the time/temp because of evaporation (refill coplin jars with dH₂O).
4. Microwave according to instructions on microwave.
5. Cool on countertop, 15 min.
6. Rinse with dH₂O
7. 1X PBS, 5 min.
8. Block in 4% Goat serum/PBS-T, 2 hours at RT.
9. For Rabbit anti-**NKX2.1** (R1231) dilute 1:1000. For guinea pig anti-**SCGB1A1** (G210, Bl. 3/24/11) dilute 1:1500, for mouse anti- **TUBA1A** (T7451, Sigma) dilute 1:1000 in blocking buffer. Spin down in µfuge for 10 min, apply to tissue and incubated at room temperature for 1 hour.

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-**NKX2.1**), Goat Alexa Fluor 555 anti-guinea pig IgG (**A21435**, Lot# 1571699, anti-**SCGB1A1**) at 1:200, Donkey Alexa Fluor 647 anti-mouse IgG **A31571**, Lot# 1549801, **Acetylated α-Tubulin**) 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 15 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 light-sealed box at 4°C.

Tissue Used: LMM.16.83A.4.30
PND03 C57BL6
Gender: Male
Weight= 2.1g

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