

Immunofluorescence **CALCA**, **SCGB1A1**, **TUBA1A1**

Purpose: Stain slides of 7µm frozen sections of PND03 C57Bl6 lungs for **CALCA**, **SCGB1A1**, **TUBA1A1** 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. Briefly equilibrate slides in Antigen Retrieval Buffer.
2. 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).
3. Microwave according to instructions on microwave.
4. Cool on countertop, 15 min.
5. Rinse with dH₂O
6. 1X PBS, 5 min.
7. Block in 4% Goat serum/PBS-T, 2 hours at RT.
8. For mouse anti-**CALCA** (C7113, **Sigma**) dilute 1:100 in blocking buffer, For guinea pig anti-CCSP (G210, Bl. 3/24/11) dilute 1:1500, for mouse anti- **TUBA1A1**(T7451, Sigma) dilute 1:1000 in blocking buffer. Spin for 10 minutes in a µfuge, apply to tissue, 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-mouse IgG1 (**A21121**, Lot# 1345050, for anti-**CALCA**) dilute 1:200, Goat Alexa Fluor 555 anti-guinea pig IgG (**A21435**, Lot# 1571699, CCSP) at 1:200, Donkey Alexa Fluor 647 anti-mouse IgG2B (**A21242**, Lot# 1148959, anti-**TUBA1A1**) at 1:200, in blocking buffer. Vortex and µfuge at full speed for 10 minutes. 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 light sealed box at 4°C.

Tissue Used: LMM.16.83A.4.19
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