

Immunofluorescence- **HOPX**, **SFTPC**, and **ACTA2**

Purpose: Stain slides of 5µm paraffin sections of PND14 lungs for **HOPX**, **SFTPC**, and **ACTA2** with antigen retrieval.

Day 1

1. Paraffin sections are placed at 60°C for 2 hours to overnight to melt paraffin. 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.
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% Donkey serum/PBS-T, 2 hours at RT.
9. For Rabbit anti-**HOPX** (**SC-30216**, Lot # C2212, Santa Cruz) dilute 1:200, For goat anti-**SFTPC** (SC-7706, Santa Cruz) dilute 1:100, for mouse anti- **ACTA2** (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 , Donkey Alexa Fluor 488 anti-rabbit IgG (**A21206**, Lot# 1387792, for anti-**HOPX**), Donkey Alexa Fluor 568 anti-goat IgG (**A11057**, Lot# 1235787, anti- **SFTPC**) at 1:200, Donkey Alexa Fluor 647 anti-mouse IgG (**A31571**, Lot# 1366514, 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. Spin down in µfuge for 10 min, apply to tissue and incubated at room temperature for 1 hour.
4. Rinse in PBS-T 3X, 5 min.
5. Dilute DAPI 1:2000 and apply to slides for 15 min.
6. Wash in PBS-T 3X, 5 min.
7. Rinse slides in 0.1M PB or TB, 3X, 5 min.
8. Add 1 drop of Prolong Gold anti-fade mounting medium (P36930).

9. Coverslip with Gold Seal Coverslip (Cat# 3422 Electron Microscopy Sciences, 22 X 22 mm).
10. Allow Prolong Gold to cure overnight at room temp in light sealed box.

Tissue Used: LMM.15.33A.6L.23
PND14 C57BL6
Gender: Female
Weight= 6.75g

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