

Immunofluorescence: SOX9, SOX2, and ACTA2

Purpose: Stain slides of 5µm paraffin sections of human lung from the HTC for **SOX9**, **SOX2**, 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. 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% Donkey serum/PBS-T, 2 hours at RT.
10. For Rabbit anti-**SOX9** (**AB-5535**, Lot # 2167153, Millipore) dilute 1:100, for mouse anti-**SOX2** (**SC-365823**, Santa Cruz, Lot# C0317) dilute 1:100, 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 , Donkey Alexa Fluor 488 anti-rabbit IgG (**A21206**, Lot# 1608521, for anti-**SOX9**) at 1:200, Goat Alexa Fluor 568 anti-mouse IgG1 (**A21124**, Lot# 1600878, for anti-**SOX2**) 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 light sealed box @ 4°C

Tissue Used:

LMH-16-D019-RLL-5B1.22

Age: 1 Day-Old

Gender: Male

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