

Immunofluorescence- **Ki-67**, **FOXF1** and **NKX2.1**

Purpose: Stain slides of 5µm paraffin sections of human lung from HTC for **Ki-67**, **FOXF1** and **NKX2.1** 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 mouse anti-**Ki-67** (**55603**, BD Biosciences, Lot# 6251774) dilute 1:100, For goat anti- **FOXF1** dilute 1:100, For Rabbit anti-**NKX2.1** (RB TTF-1 1231, Lot# 0912A) dilute 1:500 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 Apply secondary antibody , Donkey Alexa Fluor 488 anti-mouse IgG (**A21202**, Lot# 1608521, for anti- **Ki-67**), Donkey Alexa Fluor 568 anti-goat IgG (**A11057**, Lot# 1485187, for anti-**FOXF1**), Donkey Alexa Fluor 647 anti-rabbit IgG (**A31573**, anti-**NKX2.1**) 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-D043-RLL-10A2.21

Age: 21 Months-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