

Immunofluorescence- **KRT14**, **MUC5B**, and **TUBA4A**

Purpose: Stain slides of 7µm frozen slides of human lung from the HTC for **KRT14**, **MUC5B**, and **TUBA4A** 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. Antigen retrieval, pH 6.0 (times will vary according to microwave).
3. 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).
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 mouse anti-**KRT14** (**AB7800**, **Abcam**) dilute 1:100, for rabbit anti-**MUC5B** (**SC-20119**, Lot # B1003 Santa Cruz) Dilute 1:100, For mouse anti-**TUBA4A** (Acetylated Tubulin, T7451, Sigma) dilute 1:1000 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, Goat Alexa Fluor 488 anti-mouse IgG3 (**A21151**, Lot# 1563682, for anti-**KRT14**) at 1:200, Goat Alexa Fluor 555 anti-rabbit IgG (**A21428**, Lot# 1608466 for anti-**MUC5B**) at 1:200, Goat Alexa Fluor 647 anti-mouse IgG2B (**A21242**, Lot# 1148959, anti-**TUBA4A**) 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 at 4°C in a light sealed box.

Tissue Used Tissue Used:

LMH.16.D007-LLL-5C4.11

Age: 4 Years

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