

## IMMUNOFLUORESCENCE PROTOCOL

Carla Kim

For paraffin sections

1. dewax by dipping slides in jars in hood (make sure to completely submerge slides)
  - a. Process: xylene 3 min, xylene 3 min, xylene 2 min, 100% EtOH 3 min, 95% EtOH 1 min, 70% EtOH 1 min, running tap water at least 2 min  
\*\*\*\*may need to add antigen retrieval step here\*\*\*\*
2. Draw circles around sample area with pap pen
3. wash 3 X 5 min in PBS/0.2% Triton-X-100 (this solution is the base for all the following dilutions, etc)
4. block 15 min-2 hours RT in humid chamber (put slides in slidebox w/ wet kim wipes on bottom, close lid)  
for block, match with secondary antibody; if secondary antibody raised in donkey, use normal donkey serum (10% in PBS or base) to block; if raised in horse, use horse serum, etc.
5. incubate with primary antibody overnight 4 degrees in humid chamber  
(I dilute the primary antibody in PBS/0.2% Triton-X-100)
6. wash 3 X 5 min with PBS/0.2% triton-X-100
7. add secondary antibody, incubate 30 min (can do 1-2 hours @ RT), 37 degrees, humid chamber (I use Molecular Probes Alexafluors raised in donkey, diluted 1:200 in PBS)  
(e.g. donkey anti-rabbit 594 and donkey anti-goat 488; can be mixed together for incubation)  
(be careful to go step wise if one secondary was raised in goat and another is anti-goat, use the anti-goat one first for 30min, wash 3 X 5 min, then do separate incubation with other secondary)
8. wash 3 X 5 min with PBS
9. incubate with DAPI 5 min RT  
(OR, just mount with Vectastain which contains DAPI, then coverslip)
10. wash 3 X 5 min with PBS
11. coverslip with some type of antifade reagent (mowiol—or antifade from molecular probes)

for IHC:

GFP, rabbit antibody from Novus, use 1:1000, aliquots are labeled "GFP" in Carla's -20, antibody box

Sca-1 from Pharmingen

For IF:

Note, no antigen retrieval required for these, but may make it better:

CCA, 1:1000 gift rabbit antibody from Anil M. at NIH (#3975)

SP-C, 1:100 Santa Cruz #7706 raised in goat, in Carla's frig

santa cruz SP-C 13979, lot # G2803, raised in rabbit at a 1:100 dilution

CC10, 1:100 goat Santa Cruz sc-9772; sc-9772 lot #D2506 and 9773 lot #E0506  
both 1:200

SP-C, 1:100 rabbit Chemicon #3786 (raised against human SP-C, but detects mouse also)

SP-C, to detect the human protein, Chemicon #3428 1:100; this is out now, 3786 should work on human

CD34, 1:50 rat Abcam, called MEC14.7

Sca-1, 1:100 goat R&D, AF1226

CGRP, 1:5000 rabbit, Sigma

Acetylated tubulin, 1:8000 mouse, Sigma

Cytokeration, for human protein; Biogenex PU071-UP rabbit or AE1+AE3 mAB

Ecadherin, mouse anti-mouse, 610181, 1:100 with citrate buffer

BrdU: BD biosciences, (877) 232 8995, Cat# 347580, \$270 \*\*must use citrate buffer

For frozens: Inflate lung with OCT 70%/PBS 30%; embed in OCT; 6u sections

1. Warm to room temperature
2. Fix in acetone 10 min 4 degrees
3. Wash 3 x 5 min PBS
4. Go to step 3. above
5. may be better for IHC than IF

For cytospin:

1. Cytospin lung cells 600 rpm, 3min
2. Immediately draw hydrophobic circle around area with cells
3. Cytotfix/Cytoperm (BD Pharmingen) 20 min, 4 degrees
4. Go to step 2. above

\*\*\*Antigen Retrieval\*\*\*

-after dewax,

-prepare fresh each time: citrate buffer

1.0g NaOH pellets

2.1g citric acid

1 L H<sub>2</sub>O

-place slides in microwave dish or Tupperware, cover with buffer, cover with saran wrap with holes poked on top, microwave on high 6 minutes x4

-cool to room temp 10 minutes

-go to wash steps

