NEUROMICS

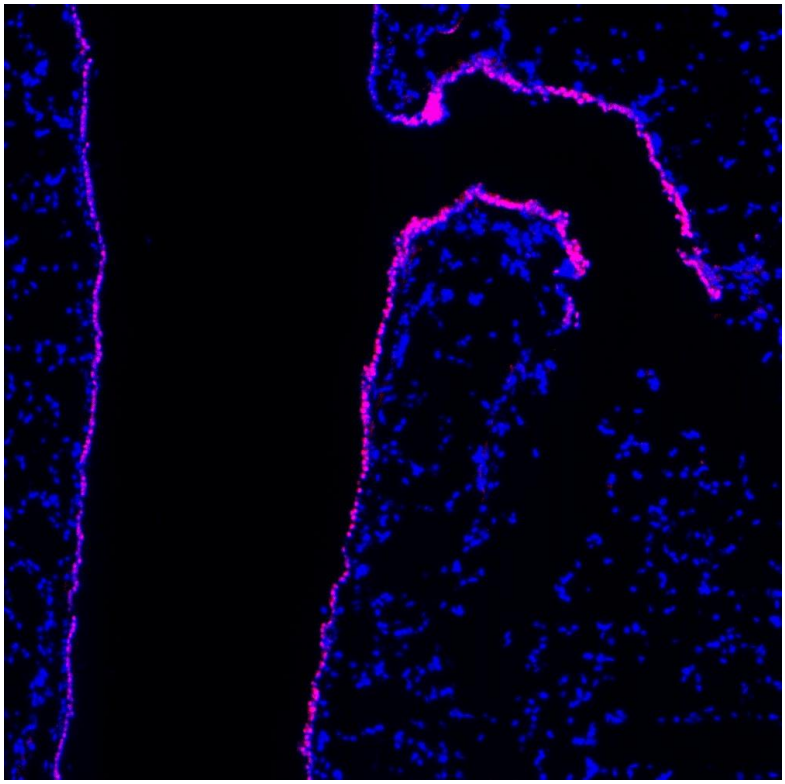
A Division of CA3 Biosciences, Inc.

Immunofluorescence staining of Cryostat Sections

Protocol and data courtesy of Monica at the University of California San Francisco

1. Prior to staining:
   Fix tissue in PFA (1 hour, 4°C)
   Wash 4 x in PBS for 30 min
   Dehydrate in 30% Sucrose overnight
   Place in 1:1 30% sucrose:OCT overnight
   Place in OCT for 3 hours, then embed in OCT
   Freeze and cut 8 um thick cryostat sections (or as needed) and mount slides

2. **Equilibrate Temp**: leave slides at RT for 1 hour
3. **Wash** 3 x 5min in PBS
   At no time from this point onwards should the slides be allowed to dry. Drying out will cause non-specific antibody binding and therefore high background
4. Apply **PAPpen** around sections to be stained
5. **Permeabilize** with 0.1% Triton X in PBS for 10 min, then wash 3x for 10 min each with PBS at room temperature
6. **Prepare Blocking reagent*** 10% Donkey Serum
   1% BSA (use 5% if blocking proves insufficient)
   Sample preparation for **1ml**: (ca 400µl per slide)
   i. 100µl Donkey Serum (DS)
   ii. 33µl BSA (30%)
   iii. 10µl MOM Blocking Reagent
   iv. 857µl PBST
7. **Block non-specific sites**
   a. Remove PBS, Place block in the dish 2 hours at room temperature
      To prevent drying out, place a damp Kimwipe on the bottom of the large culture dish (150mm) and place the slides on top
8. **Prepare the 1º Antibody**
   a. Keep all reagents on **ice**
   b. Dilute 1° Antibody with PBST (see Antibody database for concentration)
   c. Remove blocking agent and replace with the1º Ab solution
9. **Incubate** overnight in primary antibody
10. **Wash** 3 x 5min, PBST (shaker 70rpm)
11. **Dilute 2° antibodies in PBST**. (stored in Freezer 4)
    - Keep all reagents on ice
    - 2° are used at 1:250.
12. **Cover** with aluminum foil (and keep covered) as 2º Ab's fluorescent dyes are degraded by light and **incubate** for 1 hour
13. **Wash** 3 x 10min each with PBST
14. **Stain** with DAPI in PBS for 5 min
15. **Wash** 3 x 10min each with PBST
16. **Mount** with aqua mount and slip rite cover glas



SOX2 staining (dilution 1:200) of frozen OCT-embedded slides of mouse lung.