**LM046-1: small airway epithelial cell (SAEC) IF with various profibrotic stimuli**

I want to try treating small airway epithelial cells with TGFb, LPA, and fibrotic cocktail to see if any of these stimuli cause them to increase their production of HNE. I will culture them on RTC-coated coverslips for IF preparation and stain for HNE and aSMA.

**Day 1: coating coverslips and plating cells**

1. Coat five coverslips with RTC (12uL RTC + 2988uL PBS, coat 2h and wash 2x PBS)
2. Trypsinize SAECs in 1mL trypsin solution. Resuspend in 7mL SAGM and pipet into a tube containing 2mL FBS. Mix thoroughly and count cells.
   1. Count: \_\_\_\_\_0.2M/mL\_\_\_\_\_\_ Viability: \_\_\_\_\_\_78%\_\_\_\_\_
3. Spin cells down. Resuspend pellet in SAGM at 300k cells/mL.
4. Pipet 100uL suspension (30k cells) + 650uL SAGM onto each coverslip. Put the remaining cells in a flask to continue to culture.

**Day 2: treatment**

1. Make treatment media:
   1. Vehicle:

|  |  |  |
| --- | --- | --- |
| **Component** | **Volume** | **Unit** |
| HCl/BSA | 0.625 | uL |
| PBS/BSA | 2.75 | uL |
| PDGF buffer sol'n | 1.25 | uL |
| SAGM | 2.5 | mL |

* 1. TGFb: 1uL TGFb + 2mL SAGM
  2. LPA: 2uL LPA + 2mL SAGM
  3. Fibrotic cocktail:

|  |  |  |  |
| --- | --- | --- | --- |
| **Component** | **Volume** | **Unit** | **Final conc** |
| TGFb | 1.25 | uL | 5 ng/mL |
| TNFa | 0.5 | uL | 10 ng/mL |
| LPA | 5 | uL | 5 uM |
| PDGF | 2.5 | uL | 50 ng/mL |
| SAGM | 5 | mL | NA |

1. Wash cells 2x PBS.
2. Replace media with respective compositions.
3. Incubate 24h.

**Day 3: collection**

1. Quick wash with PBS x 3
2. Fix in of 3% paraformaldehyde (in PBS) for 30 min at room temp - 1ml/well (938uL 16%PFA + 4.062mL PBS)
3. Quick wash x 3 with PBS
4. Leave in PBS at 4C over weekend

**IF preparation**

1. Permeabilize with 0.2% Triton X-100 diluted in PBS for 5 minutes at room temp – 1mL per well
2. Wash x 3 for 5 minutes each with PBS
3. Make enough blocking solution for 750uL per coverslip (need this for blocking, primary, and secondary) and to make isotype
   1. 10% donkey or goat serum (whichever is the animal the secondary antibody is made in)
   2. 0.1% Triton
   3. 1%BSA
   4. PBS
4. Put 250uL of blocking solution onto each coverslip
5. Leave for 10 minutes at RT
6. While blocking, make primary antibody and isotype control diluted in blocking solution and centrifuge at 14000rmp x 5 minutes at 4 degrees
   1. Ab
   2. isotype
7. Put 250uL drops of primary antibody onto coverslips
8. Place in cold room overnight (or one hour at RT)

**Secondary**

1. Wash x 3 with PBS for 5 min each
2. Phalloidin staining
   1. Use 5uL/coverslip of phalloidin diluted in 200uL/coverslips of PBS +1% BSA (
   2. Before adding the PBS/BSA, dry the phalloidin under nitrogen (in glass tube). Remember to close the sash on the hood or brian will be upset
   3. Put 200uL drops of phalloidin solution onto coverslips
   4. Leave covered with foil for 20 minutes (to avoid bleaching)
   5. Aspirate phalloidin sol’n
   6. Wash with PBS x 3 for 5 minutes each
3. Make secondary antibody in 1:100 dilution in blocking solution (make enough for 250uL/coverslip). Centrifuge at 14000rpm for 5 minutes at 4 degrees
4. Place 250uL of antibody onto each coverslip
5. Incubate for 1 hour at room temp covered with foil
6. Wash 3 x5 min
7. After the last wash get a beaker with Mq H2O
8. Keep in mind which side cells are on and dip the coverslip into the water, then wipe off excess on rim
9. Use Kimwipe to dry the cell-free side of the coverslip
10. Place 10uL (no bubbles) of mounting medium +DAPI onto labeled slides
11. Place coverslip cell side down onto mounting medium
12. Keep slides covered and place in cold room

**Antibody info**

Primary antibodies:

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Dilution:

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Dilution:

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Dilution:

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Dilution:

Secondary antibodies:

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Dilution:

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Dilution:

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Dilution:

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Dilution:

|  |  |  |
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| **color** | **fluorophore** | **target** |
| blue |  |  |
| green |  |  |
| orange |  |  |
| red |  |  |