**Cell Culture IMCD Kidney Cells**

Materials:

DMEM F12

PS (Penicillin-streptococcus antibiotic)

FBS

PBS

Trypsin

Tissue Culture Dish

Pipette

Pipette tips (10ml, 1ml, 100ul)

G4-18 (optional)

Hemocytometer

Trypan Blue

Microcenterfuige tubes

IMCD Medium:

1. Add 10% FBS to DMEM F:12 (1:1) medium
2. Add 1% PS
3. Mix and label

Cell Culture:

1. Aspirate medium in dish
2. Wash dish with 5ml of PBS, swirl plate
3. Aspirate PBS from dish
4. Add 5mL trpsin to plate and place plate in incubator for minimum of 5 min
   1. Look at cells under microscope to verify detachment
5. Add 5mL of IMCD medium to dish and remove solution and put into 15ml tube
6. Centrifuge at 1.5 RCF for 5min
7. Aspirate supernate, make sure not to aspirate cell pellet at bottom of tube
8. Resuspend in 10mL of IMCD medium
9. Take 500ul cell suspension and place into microcentrifuge tubes
10. Add 500ul trypan blue solution (0.4% solution w/v) to tube, incubate @ RT for 5-7 min
11. Load hemocytometer with 17 ul cells on each side and count cells
    1. Count 4 corners (only count live cells, dead cells will be stained blue) and average them, then multiply by 10,000 to find cell/ml
12. Find how much cell solution you need for your selected cell seeding (Seeding #/Solution#)
13. Add amount of cell solution to plate and add (10mL-cell solution volume) of medium to dish
14. OPTIONAL: If cells are transfected and have fluorescent primary cilium add G4-18 to get 200ug/mL
15. Label and place in incubator
    1. Look at cells before placing in incubator to make sure you did add cells