Procedure for culturing and harvesting lung tissue slices

Culturing lung tissue slices:

1- In the OR, add the slices to the T25 flasks with membrane vent containing 10 ml of appropriate media (labeled or unlabeled, with or w/o inhibitor).
2- Bring the flasks with slices to the culture room and spray them with 70% ethanol before placing them in the cell culture hood. Wipe them dry. Use ethanol-resistant markers.
3- Pipet 200 μl culture media from each flask into 1.5 ml snap-cap tubes (T0 - time zero media samples). Centrifuge for 15 min at 3500 x g , 4^0C. Transfer 50 μl to tared 2 ml screw cap tube and weigh the media volume. Transfer the remaining media to a 1.5 ml screw-cap tube for long-term storage.
4- Weigh flasks in a 2-place balance inside the hood.
5- Incubate flasks on rocker inside cell culture incubator (37 ^{0}C, 5% CO_2) for 24 h.

Harvesting lung tissue slices:

1- After 24h incubation, weigh flasks (harvest 4-6 flasks at a time).
2- Place flasks on ice.
3- Using a transfer pipet, aspirate and transfer the final media into 15-ml conical tubes. Keep media on ice.
4- Wash tissue slices 2X with 5 ml cold PBS each time. Wash tissue with ice cold nanopure water. Aspirate water from the tissue and blot-dry the slice on kim-wipe.
5- Weigh the whole tissue slice on small weighing boats and record the weight.
6- If tissue slice is more than 20 mg, cut it into 3 pieces:
    a. a very small piece for histology (place it into 1.5 ml snap-cap tube containing 1 ml formalin - kept at room T);

b. a second piece weighing between 10 and 20 mg for extraction (flash freeze free floating in liquid nitrogen then place it inside a pre-LN_2chilled 1.5-ml snap-cap tube) and
c. the remaining piece for back-up (flash freeze free floating in liquid nitrogen then place it in a pre-LN_2chilled 1.5-ml screw-cap tube for long-term storage).

Record the weight of the pieces except for the histology one. If tissue slice weighs less than 20 mg, consider the experimental design and sample size to choose whether to prepare both histology and extraction sample or one or the other.

7- Centrifuge media for 15 min at 3,500 x g, 4 ^{0}C.
8- Pipet 50 µl media aliquot into 2.0 ml screw-cap tubes, weighing each sample (T24 – 24h media samples).
9- Pipet 1 ml media aliquot into 2-ml screw-cap tubes for long-term storage.
10- Transfer remaining media into a clean 15 ml conical tube for exosome isolation.

11- After 6-8 hours in formalin, replace the formalin with 70% ethanol for the tissue pieces prepared for histology.