Lung Organ Culture

Organ Preparation:

1. Dissect organs (*e.g.*, mouse embryonic lungs) into L15, 1% FBS.

2. If you are inserting a bead into the organ, it is easiest to do this prior to placing the organ on the filter. Make a small incision in the area where the bead will be placed using tungsten needles. Gently insert the bead into the incised region using either forceps or a blunted tungsten needle.

3. Place the organ into a well on top of a Nucleopore (Whatmann #110414) filter. The shiny side of the filter should be facing up. Each well should contain 2-3 ml of culture media. Place cultured organ in a 37° C CO_2 incubator.

Culture Media:
F12/DMEM 1:1
10% Hyclone FBS
1× Penicillin/Streptomycin, Glutamine

Bead Preparation:

1. Mix the bottle of Heparin acrylic beads (Sigma #H5263) very well. Remove 2 µl and place them in a dish with PBS.

2. Cut the beads with the tungsten needle (see sharpening below) into halves and quarters. The size you use depends on the application.

3. Mouth pipette the beads into a clean dish with PBS. Repeat this two more times, each time using fresh PBS, to rinse all of the azide off of the beads.

4. Place beads into an eppendorf tube and remove as much PBS as possible with a pipetman (caution: do not aspirate the beads—they will break).

5. Add 2–3 µl of recombinant protein (*e.g.*, FGF10) (1mg/ml). Let the beads incubate at R.T. for up to 4–5 hours.

6. Place tubes at 4^{O}C until use. They can be stored for 1–2 weeks but add 10–15 µl of PBS if you intend on storing them so the liquid doesn't evaporate completely.

7. Prior to use, rinse the beads 3× in PBS as done in step 3 above.

8. Place the bead in the organ as described in the organ preparation section above. The un-used beads may be stored again in PBS.