

## EXPLANT MEDIUM

### Bicarbonate Buffered Culture Medium (BCM)

Get baked 200ml beaker, 100ml baked glass cylinder, and a small stirrer from Elsa's lab. Always used autoclaved yellow tips and sterilize pipettes, and fume hood bench with alcohol before use.

In a 200ml baked beaker add:

Stock MEM (Minimum Essential Medium)	door of 4° fridge	120ml
Amino acid supplement (20ml/L)	brown box in fridge	2.5ml
Sodium pyruvate (10ml/L)	brown box in fridge	1.25ml
Vitamin supplement (10ml/L)	in -20°C freezer	1-25ml
Gentamycin (50µg/ml)	in -20°C freezer	125µl
Insulin (0.1µg/ml)	in -20°C freezer	125µl
Vitamin A (0.1µg/ml)	in -20°C freezer	25µl
Hydrocortisone (0.1µg/ml)	in -20°C freezer	25µl

- pH solution to 7.25 using glass pipettes and a rubber bulb (solution turns red at this point). Use low dose acids and bases.
- Sterilize with a 0.2µ round filter found in Elsa's cupboard. Do this in the lab downstairs in the fume hood. Make sure it is turned ON.
- use the pump system to filter the solution and put it on a setting of 4
- filtering will render the medium at a pH of 7.35
- Solution should be stored at 4°C in a baked bottle appropriately labeled. This medium is good for only 10-14 days.

### EXPLANT PREPARATION

- Biopsies are placed directly in BCM on a filter using a 6 well tissue culture plate for transport from Children's to Meakins.
- Under sterile conditions tissue is serially sectioned into pieces of approximately 0.5mm and placed on a clean 0.4 micron filter (Millipore, Bedford, MA, USA) in 2 ml of BCM. Orient filter so that the tissue is sitting inside the floating filter and is cradled in the filter with epithelium exposed and facing upward (shiny, white, convex side).
- Incubate tissue with LPS (0.001µg/ml - 1µg/ml) added directly in 2ml of BCM. Once LPS is added tap the sides of the culture dish to mix the LPS within the BCM
- Incubate tissue in 5% CO<sub>2</sub>/95% air (Dr. Mazer's 37°C incubator, lower incubator) for a specific time frame relevant to the study.

## POST INCUBATION

- Remove tissue from incubator and wipe the incubator shelf with ethanol
- Thaw out 4% paraformaldehyde (PF) by putting it in beaker with warm water
- Once thawed, place the tissue in PF for 2 hrs @ RT using the slim 10ml tubes. Use the fumehood in Elsa's lab and mark down the time the tissue was placed in PF.
- After 2 hrs transfer the biopsies in 15% PBS/sucrose for 1 hr (to make up the solution to 800ml HEPC-free PBS add 15g sucrose and then make up the solution to 1L)
- Transfer the tissue into 15% PBS/sucrose for 1 more hr
- Finally transfer the tissue into 15% PBS/sucrose and leave overnight at 4°C.

## BLOCKING THE TISSUE

- On the ICC bench put aluminium foil, liquid nitrogen in a flask, 100ml beaker with wire gauze to suspend into liquid nitrogen filled with isopentane (under the fumehood in small bottle wrapped with al foil), large forceps, cardboard cutout to block the tissue on, small pieces of al foil labeled with appropriate treatment group, labeled falcon tube to store the blocked tissue
- Suspend the beaker containing isopentane into liquid nitrogen
- Put small amount of OCT onto the white cardboard and dip into isopentane only for long enough to freeze only the ends of OCT
- Place tissue longitudinally in the middle of OCT making sure the epithelium is orientated correctly
- Suspend the tissue into isopentane to freeze the OCT completely
- Wrap the blocked tissue into aluminium foil which is appropriately labeled
- Put all the treatment groups from the same patient into the same Falcon tube and store in -80 freezer until ready to section
- Pour the left over liquid nitrogen into the tank
- Pour the left over isopentane into the jar under the hood and leave the beaker to evaporate under the fumehood