

Protocol for isolation of EC from adult liver/heart (AL)

(Protocol originally from Jackie)

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- 1) Use sterile conditions; autoclave forceps, stir bar, scissors. Or leave forceps/scissors in 70% ETOH Work in hood.
- 2) Coat plates with gelatin for at least 30min at 37°C and allow to air dry
*We didn't do it, but better do it.
- 3) Add 10ml of DMEM/antibiotics to 10cm dish.
- 4) Sac mouse with CO₂ gas and harvest organs (heart and liver in this case).
*Remove any connective tissue, white stuff but do it quickly.
- 5) Transfer liver/heart to dish (3) and transfer to hood ASAP.
- 6) Chop liver/liver with blade.
- 7) Transfer the chopped organs, add 5ml of 0.5mg/ml collagenase and mix well.
- 8) Stir organs at RT for 15min.
*Really, the incubation time depends how well the organs get homogenized. Faster for liver (usually less than 15 min) than for heart.
- 9) Use 10ml pipette to dissociate liver pieces by pipetting up and down.
- 10) Leave in 37°C for additional 10min.
- 11) Resuspend well with pipette
- 12) Spin 5 min at 1900 rpm and resuspend pellet in 10ml lysis buffer
*1900 rpm for first 2 mice organs and 1000 for the second two.
- 13) Incubate 3 min at RT

- 14) Spin and resuspend cells with media (1-2ml) and put through 70um filter
- 15) After cells have gone through filter, count cells if sorting
- 16) Spin down for 6min at 2000 and resuspend in sorting media
- 17) If plating, directly transfer the filtered cells to 6 well.
*2 hearts to one well, 2 livers to 2 wells.
- 18) Next day, remove sup, rinse (with DMEM) and add fresh media (MCDB-131 Complete w/ FBS, w/ antibiotics, Vec Technologies, Inc., Cat #MCDB-131AB)

MAEC Media

DMEM (low glucose) with D-Valine (Specialty Media cat#: D-087-B)

*DMEM (low glucose) with D-valine (replacing L-valine with D-valine is thought to inhibit the outgrowth of fibroblasts)

ECGS (final concentration of 30ug/mL) -20C jar

Heparin (final concentration of 50 ug/mL) 4C cell culture fridge

Pen/Strep Fungizone (1/100 dilution) Fungizone in -20C

Thymidine (final concentration of 2.4ug/mL) 4C cell culture fridge

Heat Inactivated FBS (15%) 56C for 30 min

Glutamine (2mM) 4C cell culture fridge

(Nathan's shelf)

Filter (0.22um) sterilize.

*Make fresh media every time. Use for one week.