SOPS for General Surgery Labs:

Title: Rat Hepatocyte Harvest

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Per I -Ready

- To make 500ml- (1000ml)
- 50ml of Per I Stock- (100ml per I)
- Dilute with 450ml distilled water- (900ml MQH2O)
- Add 0.475mg EGTA (Sigma E4378) (or to make 1000ml add .95mg).
- Stir for 20 minutes.
- pH to 7.4 (add about 0.3ml 10MnaOH).
- Bottle top filter.
- Store leftovers in cold room.

Per II FOR RAT HARVEST

- To make 100ml in hood (sterile)
- 100ml Per II in cold room
- Add 15mg of Collagenase (Sigma C5138) or from
- (Invitrogen/Gibco-17104) Add___ mg of Collagenase
- Let sit to dissolve
- Filter (bottle top).

Equipment:

(for liver harvest)

- Surgical instrument pack:
  - Large scissors
  - Small scissors
  - Forceps
  - Small curved forceps
- Sterile gauze pack
- Sutures (1pk of 2)
- 2-sterile 1cc syringe
- 16 gauge catheter
- sterile drape
- sterile gloves
- sterile tygon tubing
- betadine

- Sterile ceramic funnel
- sterile 10cm petri dish w/liver media on ice

(From 1 Rat you should get approx. 300-400million cells)

Percoll

- 180ml of precoll (Amersham 17-0891-01) add 20ml of 10X HBSS **(**Hanks Balanced Salt Solution / Gibco 14065)

Rat Hepatocyte Harvest

Procedure:

- Place Per I Ready and Per II w/collagenase in waterbath to warm up.(about 20 mins).
- Place blue pads on bench and sterile drape on top of blue pads
- Set up tubing in pump, place the tubing in the Per I and bleed through, place end of tubing back in bottle. Setting should be at 2.
- weigh rat, to anesthetize rat 0.09ml/100g body weight undiluted Nembutal stock
- place the rat in CO2 chamber for 10 seconds with gas on and 10 seconds without gas off..
- while rat is sleeping inject Nembutal.
- let Nembutal kick in and then shave the belly, wipe down with Betadine.
- place tape around rats legs, use pushpins to affix rat to board.
- cover rats with sterile drape
- open up sterile instruments, sutures, syringe and catheter without touching , place items on sterile drape
- Place ceramic funnel in Per II..
- place on sterile gloves
- Cut, opening in drape to expose rat belly
- with large scissors and forceps, cut skin in a **U,** up to liver height
- wipe off scissors and forceps with sterile gauze
- cut through abdominal wall in same fashion
- -place skin flap up out of the way ,with gauze
- push aside viscera with sterile gauze to expose hepatic portal vein
- fill syringe with 0.6ml heparin (1000 u/ml)
- using small curved forceps, loosely tie 2 sutures around portal vein
- inject heparin into inferior vena cava (IVC)

- Working as Quickly as possible, insert the catheter into hepatic portal, remove needle, connect tubing to catheter and cut IVC (small cut), tighten sutures around catheter, place sterile gauze over cut to perfuse liver better and release
- Make sure sutures are tighten, knot them twice, cut off the extra suture.
- While perfusing, **must run minimum of 50ml Per I through the liver**
- (total used 200-300ml) , flow rate of 8ml/min cut the liver out carefully with small scissors.
- When liver is free, switch the tubing from Per I to Per II, extract any air bubbles.
- Place the liver hanging above the sterile funnel. So the per II recycles.
- Run PerII through liver for 30 mins.
- then place in Petri dish and take to the hood to scrape

Harvesting the Cells (Rat)

Equipment (for scraping liver)

- Sterile gauze funnel
- cell scarper
- 2 sterile 50ml tube (if collecting NPC, 50ml tube for these).
- Liver Media

Cell are very sensitive so use care when pipetting and adding media. (**NO AIR BUBBLES**).

- using cell scraper, scrape ½ the liver into the culture dish and scrape the rest into the lid fill with media.
- Pour the cells into the gauze funnels and rinse out the Petri dishes
- Spin the tubes of cells in centrifuge @4° C at 400 rpm for 5 mins.
- Pipet off suppernant and place in NPC 50ml tubing and set on ice.
- Re-suspend cell in 30ml liver media, add 15ml percoll and invert 2 times before spinning in 4° centrifuge @ 400rpm for 10 mins.
- Suck off the dead cells and supernatant (w/vacuum flask).
- Re-suspend cells in liver media and spin 2 more times @400rpms for 5 mins each.
- Re-suspend in 30ml liver media and take (2) 10ul aliquot to count cells
- Using trypan blue at 10ul sample (cell) to 190ul trypan blue
- Place on hemacytometer, count the 2 diag. corners and add together, live cells and dead cells of each sample
- Add live cells together and divide by 2
- Add dead cells together and divide by 2
- Divide total live cells by total dead cells (this gives you your viability)

Take your live cell count and move the decimal to the left one spot and this will be your cell count times 10 to the 6^{th}.

- Divide cells in 10% calf serum liver media

Culture (Liver) Media

- Williams E (500ml)
- Pen and strep (5ml)
- L-glutamine (5ml)
- Insulin (0.16ml)
- Hepes buffer (7.50ml) Gibco 380-5630AG

10% low endotoxin calf serum for first 24 hours; 5% thereafter

Gel Coat Stock

- 100ml liver media to 1g gelatin (sigma G-1890)
  Take the 1g of gelatin and put in media, place in water bath
  After it is dissolved, filter in bottle top filter.

Gel Coating media for plating

(to make 500ml)

- 500ml Liver Media
- 5ml gel coat stock
- (1ml gel coat stock to every 100ml liver media)

10% Calf Serum Media

- (1) 500ml bottle liver media (w/hepes ,PS ,LG, Insulin)
  and 50ml Calf Serum