

## Mouse Collagen Perfusion Protocol

1. Add 25 mg Boehringer-Mannheim Collagenase A to 100 99 mL 1X Leffert's solution and stir. Place in 37 bath and stir a while longer. Add 1 mL of 2.7 %  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  to the 99 mLs and continue stirring at 37.
2. Anesthetize mouse with intraperitoneal Avertin injection at 20 uL of our stock/g body weight. If it doesn't go under in a few minutes, inject a little more.
3. Swab abdomen with 70 % ethanol. Cut from lower abdomen to chest, being careful not to cut into liver. Then make a boxlike opening in abdominal cavity to expose heart, lungs and liver.
4. Carefully inject left ventricle of heart with 1X Leffert's buffer (pre-wash) and 22-23 ga. needle attached to 50 mL-syringe to replace blood. Cut right atrium of heart and suction off outflow. Liver should blanch to yellow-brown color.
5. Carefully remove needle and inject into same hole pre-perfusion buffer (1x Leffert's plus EGTA). Inject approximately same volume as used to replace blood.
6. Remove needle and inject collagenase buffer with  $\text{CaCl}_2$ . Inject approximately same amount..
7. Remove needle and inject collagenase solution. Continue injecting collagenase until liver starts to fall apart. Continually suction off outflow from atrium during all these steps.
8. When digested, take liver out in pieces. Put into sterile dish. Add about 10 mL collagenase solution. Teast liver apart.
9. Take up liver cells and filter through mesh into 50 mL tube. Use rubber policeman to help push cells through. Leave connective tissue on mesh.
10. Spin cells at 1,000 for 5 min. Discard supt. Resuspend cells in after wash buffer (same as the collagenase buffer, Leffert's plus calcium) and spin down 3X.
11. Bring cells up to known volume in after wash buffer. Take 0.1 mL cells, 0.8 mL buffer and 0.1 mL Trypan blue and count live and dead cells (those that take up dye).

## Protein Labeling

12. Repsin cells and bring up in appropriate volume of RPMI 60 without Methionine plus 5 percent dialyzed fetal calf serum plus pen-strep. Put 500,000 viable cells on each small (35mm) plate. Starve in incubator 30 min.
13. Add 0.5 mCi of  $^{35}\text{S}$  /  $5 \times 10^5$  cells and label for 2 h. in incubator. Keep dishes in radioactive bin. Shake occasionally.

14. Harvest both adherent and floating hepatocytes (use policeman) into 15 mL tube, spin down at 1,000 for 5 min., resuspend in cold PBS, transfer to Eppendorf tubes, spin at low speed, take off PBS, and add 0.5 mL Yue's lysis buffer plus protease inhibitors and 1 mM DTT to each Eppie.

15. Rotate cells in cold room 30 ' to lyse. Spin 10 min. to clear sup. Proceed to preclearing and IP as for thymocytes.