Karen M. Lyons-UCLA
Adapted from Hausman *et. al.*
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Preadipocyte Isolation & Adipogenesis Induction

Solutions & Reagents

•DMEM/F12
•FBS
•Collagenase

•10 mg/ml (= 1.7 mM) Insulin: 9.75 mg of Insulin + 1ml of 0.22M HCl

•25 mM IBMX (isobutylmethylxanthine): 5.56 mg of IBMX + 1 ml of 0.5 N KOH

•6 mM Indomethacin: 2.147 mg of Indomethacin + 1 ml of DMSO

•10 mM Dexamethasone: 3.925 mg of Dexamethasone + 1 ml of ethanol

•HEPES Solution (1 L):

HEPES	29.8 g
NaCl	8.78 g
KCl	4.66 g
D-glucose	1.12 g
BSA	18.8 g
$CaCl_2$ (anhydrous)	0.132 g
ddH_2O	up to 1 L

Store at -20C.

•Red Blood Cell (RBC) Lysis Buffer:

NH_4Cl	8.24 g
$KHCO_3$	1 g
0.5 M EDTA (pH8.0)	20 ml
ddH_2O	up to 1 L

Filter Sterilize, store at 4C.

•Induction Medium (make fresh):

	Stock Conc.	Final Conc.	For 10 ml
Insulin	10 mg/ml	10 ug/ml	1 ul

Dexamethasone	1 mM	1 uM	1 ul
*IBMX	25 mM	0.5 mM	200 ul
*Indomethacin	6 mM	200 uM	333 ul
DMEM/F12; 10% FBS w/ ps			9.5 ml

*should be made fresh every time

•Maintenance Medium (make fresh):

	Stock Conc.	Final Conc.	For 10 ml
Insulin	*1 mg/ml	1 ug/ml	1 ul
DMEM/F12; 10% FBS w/ ps			10 ml

*Actual stock concentration is 10 mg/ml. Dilute 10 times with DMEM/F12; 10% FBS w/ ps to make 1 mg/ml

Protocol

I Tissue Collection and Digestion

1. Eithanize mice by exposure to isophorone or by cervical dislocation.

***** Following steps are performed in the biosafety cabinet *****

2. Perform a mid-line ventral incision through the animal's skin (do not cut into the peritoneal cavity).

3. Gently separate the skin and attached inguinal fat pad from the body,

4. Pool fat pads in a sterile Petri dish containing DMEM/F12 medium (might need 2 mice to obtain enough preadipocytes).

5. When dissection is complete, ransfer the tissue to a new Petri dish.

6. Mince the tissue to a very fine consistency.

7. Prepare HEPES:collagenase solution by adding 0.1 ml of 100 mg/ml collagenase to 9.9 ml of HEPES. Filter sterilize through 0.22 um filter into the Petri dish containing

the minced tissue.

8. Gently swirl the Petri dish to mix the minced tissue with the HEPES:collagenase solution and carefully transfer to a sterile plastic culture flask. Cap the flask and give one vigorous shake to get all the tissue into the collagenase solution.

9. Incubate for 1 hr in a shaking water bath at 37C, 115 rpm.

II Cell Separation

1. When digestion is complete, add 10 ml of DMEM/F12 to the flask and attach a filter membrane on a 50 ml tube. Pour the digested fat solution through the filter membrane and allow the solution to flow through by gravity. Discard undigested tissue retained on the filter.

2. Add additional DMEM/F12 to the digested fat solution to approximately 30 ml and centrifuge at 50 g (or 500 rpm) for 5 min to sediment clumps.

3. Remove the infranatant from beneath the floating cell layer, transfer to a new 50 ml tube, cap and centrifuge at 500 g for 15 min to pellet the SV cells.

4. Remove and discard all except 5 ml of the supernatant and add 10 ml of RBC lysis buffer. Cap the tube and gently vortex to resuspend the cell pellet.

5. After a 5 min incubation at room temperature, add an equal volume of plating medium (DMEM/F12 + 10% FBS). To remove endothelial cell clumps, filter the cell suspension through a 40 um filter into a new 50 ml tube.

6 Centrifuge at 500 g for 5 min, remove all but 2-3 ml of the supernatant, add plating medium up to 10 ml and resuspend the pellet by gentle vortexing.

7. Remove a small aliquot for cell counting.

III Differentiation of Primary Preadipocytes

1. Add appropriate amount of plating medium and plate the rest at a density of 2.4×10^5

cells/well (equivalent to 2.5×10^4 cells/cm^2) in 6-well plates.

2. Replace the medium next day.

3. Replace the medium every other day.

4. Two days after the cells reach confluency, differentiation is induced by the addition of induction medium.

5. After 48 h, the induction medium is replaced with the maintenance medium.

6. Replace the maintenance medium every other day. **Caution**: Do not remove all medium from lipid containing cells since they may burst if exposed to air. Typically, oil droplets become obvious 7-10 days after induction.