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Preparation of Chromaffin Cells from Mouse

adapted from MPI for biophysical Chemistry, Gottingen, Germany.
Sorensen et al., 2003, cell 114, 75-86

I. Preparation (For mice less than 2 weeks old):

1. Before starting the preparation, add the Papain into Enzyme Solution(see below) and start bubbling for ~20 min: it should be freshly activated and it is optimal if it is bubbled during the preparation of the glands
2. Put the decapitated mouse under the stereomicroscope in a sterile hood
3. Put one drop(>50ul) of Locke's Solution into a Petri dish
4. Remove the adrenal glands and put them into the drop of Locke's solution
5. Clean the surface the glands with a pair of fine tweezers under the stereomicroscope, trim off the fats and cut the glands in half
6. Filter the freshly activated enzyme solution with the 0.2µm filter and put one drop(~50µl) of it into the Petri dish
7. Put the clean glands into that drop of Enzyme Solution
8. Suck the glands and the drop of enzyme solution up with a pipette tip (1 ml blue tip) and put them into a 1.7 ml microcentrifuge tube containing 200 µl enzyme solution
9. Digest the glands for 35-45 min in a shaking bath at 37°C
10. Add 150 µl Inactivating Solution and put back into the shaking bath for 5-10 min
11. Replace the solution with 200 µl Enriched DMEM and gently triturate it with a yellow (200 µl) tip until getting a cell-suspension, usually triturate around 20 times
12. Put 50 µl suspension on the middle of each coverslip (not coated)
13. Wash the microcentriguge tube out with 200 µl enriched DMEM; add 50 µl to each drop (total amount: ~100 µl per coverslip).
14. Let the cells settle for ~15 min, usually 6 coverslips/1new-born mouse
15. Add 1 ml medium to each dish (12-well plate) and put into the incubator (10% CO_2 and 37°C)
16. Use the cells from the following day for max. 4 days

For the mice older than 2 weeks, the following steps changed:

9. Digest the glands for 20 min in a shaking bath at 37°C, triturate gently (less than 20 times), put the tube back to the shaking bath and digest another 15-20 min
10. Triturate gently and add 150 µl Inactivating Solution and put back into the shaking bath for 5-10 min, and triturate gently with a yellow (200 µl) tip. **To reduce the damage to the cell, triturate gently first and put the solution into another tube, and add some fresh enriched DMEM to the leftover glands and triturate another time, add the solution into the former tube**
11. Centrifuge 5 min with 80g and gently triturate until getting a cell-suspension, usually triturate around 20 times

Tips for the preparation

1. It is important to keep the glands intact until trituration (the most critical part is the removal from the animal)
2. To obtain excellent cells the cell density is probably the most important parameter: from an E18 embryo/newborn mouse we can seed max. 4 coverslips with reasonable cell density (~several hundred cells); if one of the glands is not intact or we have glands from younger embryos (youngest: ~E16.5), we only seed 3 coverslips

3. Cells from young animals (embryo/newborn) are less sensitive to hypoxia, but very sensitive to mechanical/chemical (e.g. osmolarity, pH) effects, therefore it is better to digest them a bit longer than triturate a gland not well digested.
4. Cells from older animals (>4 weeks), however, are more sensitive to hypoxia, therefore it is good to place them onto ice after removing from the animal and store them in ice-cold Locke's until digestion, and digest them not longer than 40 min in higher speed shaking bath.
5. It is also good to centrifuge the cell suspension from older animals(80g for 5min) and discard the supernatant, in order to get rid of debris etc.
6. In order to get more healthy cells, it is very helpful to triturate gently (you can still see the glands) and put the solution onto the coverslips, then triturate the left glands another time and add the solution to the coverslips.

II. Solutions

Locke's solution

154 mM NaCl
5.6 mM KCl
0.85 mM NaH_2PO_4
2.15 mM Na_2HPO_4
10 mM D-glucose
pH 7.4, 300 mOsm, filter

Enriched DMEM

500 ml DMEM 4.5g/l glucose, special
0.5 ml Pen./Strep. (10000unit/10mg)
5 ml Insulin-Transferrin-Selenium-X (ITSX)

Enzyme Solution

250 ml DMEM
50 mg L-Cysteine (free base)
2.5 ml 100 mM $CaCl_2$
2.5 ml 50 mM EDTA
aliquot and freeze until use
before use: add 20-25 units/ml Papain
bubble with carbogen (5% CO_2 in O_2) for 10-20 minutes, filter

Inhibitor Solution

225 ml DMEM
25 ml FCS or FBS, heat inactivated
625 mg Albumin (Bovine, Fraction V)
625 mg Trypsin-Inhibitor (Type II-O, Chicken Egg)
aliquot and freeze until use; filter before use

III. Sources

	Company	Cat. NO.	Comments
DMEM	Invitrogen	12430-054	for Enriched DMEM
DMEM	Invitrogen	21068-028	for Enzyme Solution
ITSX	Invitrogen	51500-056	
FCS	Invitrogen	10500-064	
Papain	Worthington	3126	
Albumin	Sigma	A-9418	
Trypsin-Inhibitor	Sigma	T-9253	
L-Cysteine	Sigma	C-7352	free base