Adrenal Chromaffin Cell Cultures

For rat-derived _cultures, adrenal glands from 7- to 12-day-old Sprague Dawley rats _are dissected in ice-cold Hanks Balanced Salt Solution (HBSS).

1. Animals are decapitated (anaesthetize the animals with Ketamine, Ketaset Fort Dodge NDC-0856-2013-01 if decapitation is not an approved protocol).

2. Decapitate and pin the body belly-down. Spray with 70% ethanol.

3. Cut the skin along the spinal column (it's easier starting from the neck) and pull it out to both sides, using a scissor to separate the skin from underlying tissue. The back of the body is now open.

4. Grab the vertical column with forceps and pull it up while cutting along both sides through the ribs. It's better to cut both sides simultaneously (alternating cuts on each side as you work back). Eventually you will see the diaphragm. At this point, open the scissors and place onto the diaphragm approximately 1/3 of the way up from the bottom. Pull the spine up while holding the diaphragm down. The abdominal cavity should tear open exposing, among other organs, the two kidneys with the adrenal glands on top. These should be readily visible.

5. Remove the glands with fine forceps (curved are the best, pinch off the tissue under the glands and pull up) and put them into ice-cold HBSS (Ca2+/Mg2+ free).

6. Adrenal glands are encased by adipose tissue and a capsule. Remove the capsule (using two fine forceps, try to pull the capsule open like a bag of potato chips, then holding the capsule with one tweezer, use the other to "roll" off the gland). Cut the adrenal glands in half (or thirds depending on size of glands).

7. After several washes with HBSS (use a sterile plastic transfer pipette), the _tissue is digested with **collagenase IA** solution in 10ml HBSS, Ca2+/Mg2+-free, (250-350U/ml, Worthington) for about 30-45 _min at 37°C with stirring. Stop the digestion as soon as the solution starts to turn cloudy.

8. The digested tissue is rinsed with HBSS _and triturated gently in a solution containing 1% heat-inactivated _fetal bovine serum and 0.02% DNase I. For trituration use large bore tech-tips, and if needed, medium bore tech-tips (please refer to our "Ventral Midbrain Cultures" protocol for the instruction on how to prepare the tech-tips).

9. Dissociated cells are _centrifuged at 1000 g for 3 min to form a pellet and then _resuspended in a culture medium comprised of DMEM, 10% fetal bovine_ serum, 50 U/ml penicillin, 50 μg/ml of streptomycin, and 2mM _Glutamine.

10. The cell suspension is plated onto poly-D-lysine- and _laminin-coated glass wells in 50 mm dishes (cells from 5 rat 10day-old pups onto_ 16 dishes) and, after 2 hr, the dishes are flooded

with the _culture medium (3ml per dish). Please refer to our "Ventral Midbrain Cultures" protocol for the instruction on how to prepare and coat the dishes .

11. Cells are maintained in a 5% CO2 incubator at_ 37°C.

All measurements are conducted between 1 and 7 post-plating_ days.

Suggestions for plating density for rat and mouse CC cultures:

3 10-day-old rat pups – 8 dishes
5 10-day-old rat pups – 16 dishes

2 adult mice - 12 dishes
10 10-day-old mouse pups - 12 dishes

CC Media 200ml:

2ml L-Glutamine
240ul Pen-Strep
20ml Fetal Bovine Serum, heat-inactivated
180ml DMEM

For 10ml trituration solution:

10ml HBSS
30ul DNase stock (final concentration 0.02%)
100ul Fetal Bovine Serum, heat-inactivated

Catalog #'s:

L-Glutamine – 200mM, Sigma # G2150
Pen-Strep - Sigma # P0781
Fetal Bovine Serum, heat-inactivated - Gibco # 16140-063
DMEM - Sigma # D5546
HBSS – Gibco # 14175-095
Collagenase Type I – Worthington # CLS-1
DNase I – Worthington # 2006

Preparation of DNase I stock solution:

Reconstitute with HBSS to a concentration of 2,000 U/ml
(for example: a vial with 20 mg and 3,364 U/mg is reconstituted with 33.64 ml HBSS).
Store as 500ul aliquots at -80'C.