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Adrenal Chromaffin Cell Cultures

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ABSTRACT

This protocol is about culturing of adrenal chromaffin cells from rats.

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).

ATTACHMENTS

Adrenal Chromaffin Cell Cultures.pdf

DOI

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rat-derived cultures, cell culture, adrenal, chromaffin, rat

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GUIDELINES

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

MATERIALS TEXT

Materials:

Anaesthetic (if decapitation is not an approved protocol): Ketaset® KETAMINE, FORT DODGE® (#NDC-0856-2013-01)

L-Glutamine solution, 200 mM **Sigma Aldrich Catalog #G2150**

Penicillin-Streptomycin **Sigma Aldrich Catalog #P0781**

Fetal Bovine Serum, qualified, heat inactivated, United States **Thermo Fisher Scientific Catalog #16140063**

DMEM - low glucose **Sigma Aldrich Catalog #D5546**

HBSS, no calcium, no magnesium, no phenol red **Thermo Fisher Scientific Catalog #14175095**

Collagenase Type 1 **Worthington Biochemical Corporation Catalog #LS004197** (Code CLS-1)

Deoxyribonuclease I **Worthington Biochemical Corporation Catalog #LS002006**

CC Media (200 mL):

2 mL L-Glutamine

240 µl Pen-Strep

20 mL Fetal Bovine Serum, heat-inactivated

180 mL DMEM

Trituration solution (10 mL):

10 mL HBSS

30 µl DNase stock (final concentration 0,02%)

100 µl Fetal Bovine Serum, heat-inactivated

Preparation of DNase I stock solution:

Reconstitute with HBSS to a concentration of **2000 U/mL** (for example: a vial with **20 mg** and **3364 U/mg** is reconstituted with **33.64 mL HBSS**). Store as **500 µl aliquots** at **-80 °C** .

SAFETY WARNINGS

For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).

1 Animals are decapitated (anaesthetize the animals with Ketaset® KETAMINE, FORT DODGE® #NDC-0856-2013-01 if

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decapitation is not an approved protocol).

2 Decapitate and pin the body belly-down. Spray with [M]**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 (Ca^{2+}/Mg^{2+} 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** 1h 15m solution in **10 mL HBSS , Ca2+/Mg2+-free, (250-350 U/ml, Worthington)** , for about **00:30:00** - **00:45:00** 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 [M]**1 % heat inactivated fetal bovine serum** and [M]**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.

Postnatal ventral midbrain dopamine neuronal culture protocols
by Megan Freund
PREVIEW RUN

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Dissociated cells are centrifuged at **1000 x g, 00:03:00** to form a pellet and then resuspended in a culture medium comprised of DMEM, [M]**10 % fetal bovine serum** , [M]**50 U/mL penicillin** , [M]**50 µg/mL streptomycin** , and [M]**2 Milimolar (mM) Glutamine** .

10 The cell suspension is plated onto poly-D-lysine and laminin-coated glass wells in 50 mm dishes (cells from 5 rat 10-day- 2h

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old pups onto 16 dishes) and, after **02:00:00** , the dishes are flooded with the culture medium (**3 mL per dish**).

Please refer to our “Ventral Midbrain Cultures” protocol for the instruction on how to prepare and coat the dishes .

Postnatal ventral midbrain dopamine neuronal culture protocols
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Cells are maintained in a **5% CO_2 incubator** at **37 °C** .

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

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