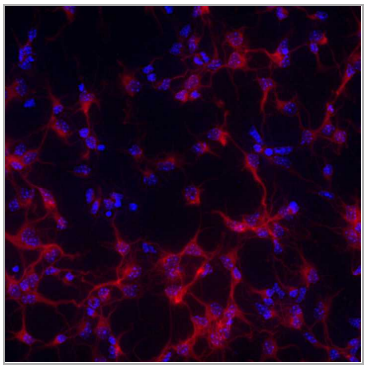

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We use this protocol and it's working

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# Isolation and Culture of Mouse Cortical Neurons

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## ABSTRACT

We provide a detailed protocol to isolate and culture primary cortical neurons from the cortex of mice at embryonic day 15.5.

## GUIDELINES

This protocol is designed to generate primary cultures of cortical neurons from the cortex of E15.5 mice. The protocol is designed based on the dissection of brain tissues from 4 embryos, but it can be scaled up as needed.
The protocol is structured in two sections:
1. Tissue Dissection
2. Culture plating and maintenance

The length of the protocol is ~9 days
Please Note: Breeding and euthanasia of all animal work should be performed in accordance with an institutionally approved animal care and use protocol.

## MATERIALS

### REAGENTS

**HBSS-G (HBSS/0.6% glucose)**
Hanks' balanced salt solution (1X HBSS) (Invitrogen #14175095)Ca2+ and Mg2+-free
Dissolve 6g glucose (Sigma #G7528-1KG) in 1L 1X HBSS
Filter (0.22um) (Millipore S2GPU05RE)
The HBSS can be stored at 4C for several weeks.
Note= Add 1x gentamicin (1000X= 50mg/ml) (Gibco # 15750-060) to the HBSS used in TC room

**Poly-D, L-lysine hydrobromide (PDLL) (Sigma #P7280)**
Dissolve 5mg in 500ml PBS (0.01mg/ml)
Filter and store at 4C

**Trypsin, 0.25% (1X) (Gibco #25200-056)**
Dilute 1:1 with HBSS-G before use.

**Deoxyribonuclease I (DNAse I) from bovine pancreas (Sigma #D5025, 15KU)**
STOCK 10X 1mg/ml in HBSS. Aliquot and store @ -20C. Use at a final concentration of 0.1mg/ml.
Thaw **DNAse** on ice and do not vortex

**NEUROBASAL COMPLETE (NBC)**
Neurobasal medium (Invitrogen 21103-049)
1x B27 supplement (50X, Invitrogen 17504044)
1x GLUTAMAX (100X) (Stored at room temp in TC) (Gibco #35050-061)
1x pen/strep 10,000U (200X) (Gibco #15140-122)
Store @ 4C.

**SOYBEAN TRYPSIN INHIBITOR**
Invitrogen 17075029 or Sigma #T9003
Dissolve soybean trypsin inhibitor in HBSS-G or Neurobasal Medium at a final 0.4mg/ml concentration. Add 20mg in 50 ml. Filter with 0.22um filter and store @ 4C.

**B-27 serum-free supplement (Invitrogen #17504-044)**

**Glutamax-I supplement (Invitrogen #35050-061)**
It is supplied as a 100X (200 mM) solution. Make aliquots inside the hood and store at 4C or 20°C for longer storage.

**Cytosine beta-D-arabinofuranoside (Ara-C, Sigma #C1768)**
Dissolve in H202. Make aliquots and store at -20C

**Trypan Blue:** (Life technologies #1394110)

**EQUIPMENT:**
Cell counter: (Biorad, model:TC 20 automated cell counter)
Counting slides: (Biorad, #145-0011)
10cm Culture Dish: (Genclone, #25-202)
Centrifuge: (Eppendorf, Centrifuge 5810R)
15mL tube: (Olympus, 29-103)
Advanced Cell Strainers, 40µm, 50 Cell Strainers/Unit: (Genesee, #25-375)

BEFORE START INSTRUCTIONS

1. Coat 10cm dishes with Poly-D-Lysine . Keep the dishes in the tissue culture incubator at 37C for 1 hour.
2. Rinse the dishes twice with sterile H2O.
3. Leave the dishes drying in the TC incubator while isolating neurons.

Prepare reagents (details in materials)

Complete Neuralbasal medium (NBC)

1. HBSS-G
2. 0.25% Trypsin in HBSS-G
3. Soybean Trypsin Inhibitor

(Optional) Filter reagents with 0.22um filters

Tissue dissection

Before starting:

- Spray down the dissection bench with 70% isopropanol.
- Prepare dissection tools
- Prepare an ice bucket for chilling dissection dishes.
- Place on ice 15ml tubes with 14ml HBSS-G for cortices isolated from 4 embryos
- Pre-warm NBC media and 1:1 0.25% Trypsin/ HBSS-G and warm HBSS-G at 37°C in a water bath. Use 5ml of Trypsin/HBSSG for each 15ml tube and 15ml of media for 4 embryos.
- Thaw 10X DNAse 1 (1mg/ml) on ice (can remove from -20C on step 6). Dilute 1:10 in Soybean Trypsin Inhibitor medium before use (0.1mg/ml final concentration). Use 1ml of DNAse/Trypsin inhibitor medium for each 15ml tube

## Tissue Dissection

1h

1 Collect the embryos in a 10 cm Petri dish with ice-cold HBSS-G and cut the heads. Keep the Petri dish on ice while dissecting.

2 Using a microscope, insert the tip of tweezers into the eyes to keep the head fixed, cut up from the cranial floor to the nose, open the skull like a book, and lift the cortices with tweezers. Using a clean 10cm Petri dish with ice-cold HBSS-G for each 3-4 embryos reduces excessive contamination with blood.

3 Dissect the cortex by removing hindbrain and olfactory bulbs, and trim away meninges. Use

tweezers to lift the meninges without puncturing the cortex tissue.

4 Transfer the cortex to the 15ml tube containing 14ml of HBSS-G on ice. 4 heads per 15mL tube

## Culture Plating and Maintenance

2h

5 After dissections are complete, work inside the biosafety cabinet. Aspirate the HBSS-G, leaving as little of the dissection medium as possible without disturbing the tissue or exposing it to air. Add ~5ml/tube of warm HBSS-G/trypsin. Incubate for 20 minutes at 37C in a water bath. Invert the tube every 5 minutes.
Meanwhile, dilute the DNAse I 1:10 in warm trypsin-inhibitor solution.

6 Remove the tube containing trypsin and pieces of tissue from the water bath. Spray it with 70% ethanol and wipe it down before moving it into the hood. Aspirate trypsin solution and add ~5ml warm HBSS-G. Let the pieces of tissue settle to the bottom of the tube for 1-2 minutes.

7 Aspirate HBSS-G, leaving as little of the dissection medium as possible without disturbing the tissue or exposing it to air. Add 1ml NB/trypsin-inhibitor/DNAse I.

8 Triturate tissue pipetting up and down using a 1ml tip. Avoid introducing bubbles. Using the fewest strokes possible to dissociate the tissue is important to improve cell survival. Triturating over 15 times seems to reduce neuron survival. Let any chunk of non-dissociated tissue sink at the bottom of the tube for 1-2 minutes.

9 Transfer dissociated neurons to a new 50ml tube with a nested 40um mesh pre-rinsed with NBC (~2-3mL) and leave the chunks at the bottom. After transferring neurons, do a post-rinse of the 40um mesh with NBC.

10 Spin cells for 5 minutes at 1200 rpm at RT. Resuspend the pellet in warm culture medium (NBC). If a blood ring is present in the cell pellet, avoid carrying over the blood. Use a total volume of ~ 1ml/embryo.

11 Count the live cells with trypan blue.

12 Plate the cells in desired culture dishes. Use ~50% of final volume of NBC for initial plating. Incubate at 37 in the incubator for 5 minutes.

13 Aspirate the initial culture media and replace it with fresh warm NBC (final volume). This step helps the removal of floating dead cells and debris. For example, for a 10 cm dish, use 10ml as final volume.

14 Change 50% of culture media every 3 days. Add AraC at a final concentration of 4uM starting from day 3. Cultures are maintained until DIV 9. We noticed that after DIV9 the viability is reduced.