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Mouse Cortical Neuron isolation

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We use this protocol and it's working

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Abstract

This protocol describes a detailed method for isolating and preparing cortical neurons from E16-17 mouse embryos.

Materials

1. **Hanks' Balanced Salt Solution (HBSS)** without Mg++ and Ca++ (Life Technologies 14170161)
2. **MgCl2•6H2O** (Honeywell Fluka M9272)
3. **HEPES free acid** (Sigma H4034)
4. **Kynurenic acid** (Sigma K3375)
5. **NaOH pellets** (Sigma S5881)
6. **Ovomucoid trypsin inhibitor** (Worthington Biochemical)
7. **Bovine Serum Albumin (BSA)**
8. **Papain**
9. **DNase** (Deoxyribonuclease)
10. **1X Dissociation Media (DM)**
11. **Neurobasal medium (plain and complete)** (Life Technologies 21103049)
12. **B27 supplement** (Life Technologies 17504044)
13. **Penicillin-streptomycin** (Life Technologies 15140122)
14. **GlutaMax** (Life Technologies 35050079)
15. **Poly-D-lysine** (Sigma-Aldrich P7405-5MG)
16. **Laminin** (Invitrogen 23017015)
17. **Isoflurane** (for mouse euthanasia)
18. **70% ethanol**

Equipment:

1. **Dissection microscope**
2. **Petri dishes (15 cm, 10 cm, and black-bottom dissection dish)**
3. **Conical tubes (15 mL and 50 mL)**
4. **Pipetteman (P1000) and corresponding pipette tips**
5. **Cell strainer (0.4 µm, BD Falcon REF 352340)**
6. **Diapers (for covering the dissection area)**
7. **Beaker for ethanol (with paper towel at the bottom)**
8. **Bucket of ice**
9. **Scissors and forceps (55 Inox forceps and crummy forceps)**

Preparation for Plating

1 Prepare the solution:
Concentrations
- **Poly-D-lysine:** 5 mg/mL (1:50 dilution; Sigma-Aldrich P7405-5MG)
- **Laminin:** 0.5-2.0 mg/mL (1:250 dilution; Invitrogen 23017015)

1.1 To prepare 50 mL of the coating solution:
- Add 1 mL of Poly-D-lysine.
- Add 200 µL of Laminin.
- Add 48.8 mL of filtered water.

1.2 **Coating volumes** for type of plate:
- 15 cm: 15 mL
- 10 cm: 10 mL
- 6 well: 1.5 mL
- 12 well: 500 uL
- 24 well: 500 uL

1.3 Coat plates the night before. Overnight 37 °C

Preparation of Dissociation Media (DM) – 10X Stock Solution

2 **Reagents Needed:**
- Hanks' Balanced Salt Solution (HBSS) without Mg++, Ca++
- Magnesium chloride hexahydrate (MgCl2•6H2O)
- HEPES free acid (Sigma H4034)
- Kynurenic acid (Sigma K3375)
- Sodium hydroxide pellets (NaOH, Sigma S5881)

2.1 Preparing 500 mL of 10X DM Stock:
a. Pour 450 mL of HBSS (without Mg++ and Ca++) into the glassware.
b. Place the glassware on a hot plate, setting the temperature to approximately level 2 to start heating.
c. Add 10.15 g of MgCl2•6H2O to reach a final concentration of 100 mM MgCl2.
d. Add 11.92 g of HEPES free acid to achieve a final concentration of 100 mM HEPES.
e. Add 1182 mg of kynurenic acid for a final concentration of 10 mM kynurenic acid.
f. Heat the solution to 65°C to help dissolve the kynurenic acid.
g. Allow the solution to cool, and adjust the pH to 7.2 by monitoring the color change.
h. Bring the total volume up to 500 mL by adding the remaining HBSS.
i. Aliquot the solution into 27.5 mL portions and store at -20°C.

Neurobasal Complete Media (NBc)

3 ▪ **Reagents:**
- o B27 (Life Technologies 17504044)
- o Pen/strep (Life Technologies 15140122)
- o Glutamax (Life Technologies 35050079)
- o Plain neurobasal medium (Life Technologies 21103049)

3.1

	Reagent	500 mL	250 mL	100 mL
	B27	10 mL	5 mL	2 mL
	Pen/Strep	5 mL	2.5 mL	1 mL
	GlutaMax	5 mL	2.5 mL	1 mL
	Plain Neurobasal Medium	500 mL	250 mL	100 mL

Preparing Dissociation Reagents

4 Label four 15 mL conical tubes: Heavy, Light, DNase, and Papain.

4.1 Weigh 0.06 g of ovomucoid trypsin inhibitor (Worthington Biochemical) and add to the Heavy tube. Add 0.06 g of BSA to the Heavy tube.
Ovomucoid is a general protease inhibitor that can inhibit papain.

4.2 Add ~2.5-3.0 mg of DNase to the DNase tube.

4.3 Add a few grains of L-cysteine to the Papain tube

4.4 In the tissue culture hood, add 6 mL of sterile 1X DM to Heavy. Warm the Heavy tube in a water bath until the ovomucoid and BSA dissolve. Adjust the pH of Heavy with sterile filtered 1N NaOH (~20 µL).

4.5 Prepare Light as a 1:10 dilution of Heavy by adding 1 mL of Heavy to 9 mL of 1X DM. Invert to mix.

4.6 Add 5 mL of sterile 1X DM to the Papain tube. Add 100 µL of Papain suspension (Sigma 10108014001) to the Papain tube and warm the mixture in a water bath. Sterilize the Papain with a 0.2 µm filter and transfer it into the DNase tube. (Papain/DNase)

4.7 Keep Heavy, Light, and Papain/DNase tubes in the warm water bath.

Brain Dissection:

5 Isolate the cortex:

5.1 Take dissection tools out of the 70% ethanol beaker and dry them. Lay the mouse supine and spray the body with 70% ethanol.

5.2 With the crummy forceps, lift the stomach and make a cut with scissors. Avoid letting the sacs touch the fur. Carefully separate the sacs from other organs and transfer them to the 15 cm dish.

5.3 Tear open the amniotic sacs with two 55 Inox forceps. Once the embryos are free from the sac, sever the heads with the forceps and move them to the lid of the 10 cm dish.

5.4 Under the dissection microscope, pierce the eyes with forceps and peel away the skull. The brain will appear white without blood vessels. Gently scoop out the forebrain and transfer it to the dissection dish using a P1000 pipetteman with a cut-off tip.

5.5 Separate the lobes from the midbrain and carefully remove the meninges. Isolate the hippocampus and transfer the cortices to a tube with cold 1X DM.

Dissociation Process

6 Dissociation Steps:

6.1 Remove as much 1X DM as possible from the cortical tissue and add 6 mL of Papain/DNase mixture. Incubate for 10 minutes, stirring every 2-3 minutes.

6.2 Wash the cortices three times by adding 3 mL of Light solution. Allow the cortices to settle each time before removing the Light.

6.3 Wash once with 4 mL of Heavy solution, waiting for the cortices to settle before discarding the Heavy.

6.4 Wash twice with 3 mL of cold Neurobasal Complete (NBc), allowing the cortices to settle after each wash before removing the NBc.

6.5 Add 1 mL of Neurobasal Complete (NBc) using a P1000 pipetteman. Mix the solution by pipetting up and down 10 times, ensuring that the cortices are drawn into the pipette tip during the process.

6.6 Place a 0.4 µm cell strainer onto a 50 mL conical tube. Allow the undissociated cortices to settle, and then use a P1000 pipetteman to add the supernatant to the cell strainer.

6.7 Wash the cell strainer with 3 mL of Neurobasal Complete (NBc).

6.8 Repeat this process a total of three times

6.9 For the fourth wash, add 1 mL of NBc, then transfer the entire contents of the tube to the cell strainer.

6.10 Wash the cell strainer again with 3 mL of NBc

Plating

4h

7 Count cells.

7.1 Wash coated plates with sterile water two times and one time with Neurobasal plain.

7.2 **Plate cells accordingly:**

Number of cells / plate type:
15 cm: 25-30 million cells / plate
6 well: 1-1.5 million cells / well
12 well: 0.5 – 0.75 million cells / well
24 well: 90,000 cells / well.

7.3 Place cells in incubator 37 °C 5% CO2.