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PRIMARY GLIA ISOLATION AND CULTURE PROTOCOL

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We use this protocol and it's working

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Abstract

This protocol details the isolation of primary glia from cortex.

Materials

OPC Media:

	A	B
	Apo-transferrin	20S + 50 µg/ml
	Insulin	5 µg/ml
	Sodium selenite	30 nM
	D-biotin	10 nM
	Hydrocotisone	10 nM
	PDGF-AA	20 ng/ml
	bFGF	20 ng/ml

Troubleshooting

Primary Glia Isolation From Cortex Protocol

2d 9h 44m

1 **Before dissection:**

1.1 Clean and autoclave all dissection tools (scissors, forceps, spatulas, razor blades) prior to use.

1.2 Prepare dishes or plates.

1h

- Minimum of 01:00:00 in 37 °C incubator.

1.3 Have solutions warmed, equilibrating, and prepared prior to starting dissection (plating media, digestion solution, digestion inhibition solution).

- Sterile filter digestion and inhibition solutions prior to use.

1.4 Flame polish autoclaved 9″ Pasteur pipettes.

2 **Dissection:**

2.1 In laminar flow hood: have aluminum foil for mice, Kimwipes or paper towels for dissection, tools, ice bucket and Brain Bits Hibernate A (BB HA).

2.2 Begin dissection (steps may be done simultaneously on 6-8 pups or sequentially on each pup).

- Remove tools from alcohol
- Decapitate pup/s with scissors
- Use razor to make a mid-sagittal incision only penetrating the skin
- Use razor to make a small mid-sagittal incision in the skull, then press down hard hemisecting the brain and skull. Push apart.
- Dip blunt dissecting spatulas into the wash solution. Scoop out brain hemisphere, severing the olfactory bulb for ease
- Separate and isolate cortex.
- Place in chilled BB HA solution
- Using fine forceps, remove meninges from cortical surface

2.3 Keep On ice until ready to place cortex into warmed and sterile filtered digestion solution.

3 **Digestion:**

3.1 Using 10 mL serological pipette, transfer cortices from BB HA to digestion solution.

3.2 Incubate in 37 °C water bath for 00:10:00 - 00:15:00, with intermediate mixing. 15m

3.3 During this time:

- Ensure plates/dishes are ready
- Prepare trypan blue Eppendorf tube (150 µL TB + 50 µL cells) to count

4 **Inhibition + Triturate:**

4.1 Following digestion incubation, gently remove cortices with 10 mL serological pipette and place into 15 mL conical tube.

4.2 Wash cortices 3x with inhibition solution (3-4mL/wash).

4.3 Then add final 4 mL - 5 mL inhibition solution and triturate cortices gently using fire polished pasteur pipette.

4.4 Once triturated, allow any undissociated tissue to sink to the bottom, gently transfer remaining suspension to fresh 15 mL tube.

4.5 Pass remaining supernatant through 70 µm and 40 µm cell strainers to isolate single cell suspension.

4.6 Pull 50 µL aliquot for counting, then centrifuge at 300 x g, 4°C, 00:04:00 . 4m

5 **Count cells:**

5.1 Make up the 200 µL (1:4 dilution of cells) trypan blue mixture, load 10 µL to hemacytometer, and count 4 quadrants.

5.2 Calculate desired concentration of cells/mL.

6 **Plate cells:**

6.1 Dilute cells with appropriate amount of pre-equilibrated plating media, (20S: DMEM, 1 mM Sodium pyruvate, Glutamax, Penicillin-streptomycin and FBS-20%) to get desired cell concentration.

6.2 Plate 12,000,000 cells in each matrigel coated T75 flask.

7 **Microglia isolation and culture:**

7.1 To obtain primary microglia, shake confluent T75 flask at 220 rpm, 37°C, 01:00:00 .

7.2 Centrifuge suspended microglia at 300 x g, 00:05:00 and resuspended in 20S plating medium followed by filtering through 70 µm cell strainer. 5m

7.3 Plate cells at desired concentration 48:00:00 prior to experiment. 2d

8 **Oligodendrocytes isolation and culture:**

8.1 After microglia have been removed from T75 flasks, replace media and shake at 220 rpm, 37°C Overnight . 8h

8.2 Filter suspended oligodendrocytes using 40 µm cell strainer and centrifuge at 200 x g, 00:10:00 . 10m

8.3 Resuspend cell in OPC media.

OPC media:

	A	B
	Apo-transferrin	20S + 50 µg/ml
	Insulin	5 µg/ml
	Sodium selenite	30 nM
	D-biotin	10 nM
	Hydrocotisone	10 nM
	PDGF-AA	20 ng/ml
	bFGF	20 ng/ml

8.4 Plate cells 7-10 days prior to experiment.

9 **Astrocytes isolation and culture:**

9.1 After microglia and oligodendrocytes have been removed from T75 flasks, wash the remaining attached cells (astrocytes) twice with PBS detach using 0.25% Trypsin-EDTA, add 5 ml NbAstro media and filter through 40 µm cell strainer and centrifuge at 300 x g, 00:10:00 . 10m

9.2 Resuspend pellet in NbAstro media and filter through 70 and then 40 µm cell strainer.

9.3 Plate astrocytes at 800,000 or 400,000 cells per well for 2-4 days before experiment for biochemical or immunocytochemical analysis, respectively.