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# (3) PRIMARY GLIA ISOLATION AND CULTURE PROTOCOL

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We use this protocol and it's working

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## **Funders Acknowledgements:**

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## **Abstract**

This protocol details the isolation of primary glia from cortex.

## **Materials**

### **OPC Media:**

Α	В
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

- 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
	solutio	on.	

### 3 Digestion:

- 3.1 Using 10 mL serological pipette, transfer cortices from BB HA to digestion solution.
- 3.2 Incubate in \$\mathbb{8}\$ 37 °C water bath for \&\infty\$ 00:10:00 - \&\infty\$ 00:15:00 , with intermediate mixing.



### 3.3 During this time:

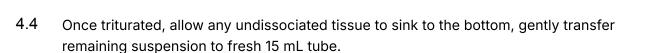
- Ensure plates/dishes are ready
- Prepare trypan blue Eppendorf tube ( Δ 150 μL TB + Δ 50 μL cells) to count

#### 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 - 4 5 mL inhibition solution and triturate cortices gently using fire polished pasteur pipette.



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



- 4.6 Pull  $\perp$  50  $\mu$ L aliquot for counting, then centrifuge at  $\bigcirc$  300 x g, 4°C, 00:04:00 .
- 4m

- 5 **Count cells:**
- 5.1 Make up the  $\perp$  200  $\mu$ L (1:4 dilution of cells) trypan blue mixture, load  $\perp$  10  $\mu$ L 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 \$\(5)\) 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 5 220 rpm, 37°C Overnight



8.2 Filter suspended oligodendrocytes using 40 µm cell strainer and centrifuge at

10m

**₩** 200 x g, 00:10:00 .

8.3 Resuspend cell in OPC media.

## **OPC** media:

А	В
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 **3**00 x q, 00:10:00





- 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.

