Microglia isolation from mouse and culture (modified from UCSD)

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This protocol details microglia isolation from mouse and culture (modified from UCSD).

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Microglia isolation, UCSD, mouse

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General notes

- (Maximum 6 mice per preparation; protocol described below for 6 brains; ~300,000 cells per 7/week old).
- For high yield better use really young mice, let's say with P7-14 you may get a lot more than above!
- Mechanical dissociation >> enzymatic dissociation.
- Keep everything at 🌡 **4 °C** .
- In vitro: 150k cells/ well (one brain/well) in a 48 well plate).
- Will get much more microglia with pups à use microglia from pups to test antibodies and for general practice.

☒ HBSS (1x) **Gibco - Thermo Fisher Catalog #14175-095**

☒ 2 ml polytetrafluoroethylene pestle (Wheaton) **Raptor supplies Catalog #358026**

☒ Corning™ Falcon™Round-Bottom Polypropylene Tubes **Fischer Scientific Catalog #352059**

☒ Falcon® 5 mL Round Bottom High Clarity PP Test Tube with Snap Cap Sterile **Corning Catalog #352063**

☒ 2 ml glass mortar (Wheaton) **Raptor supplies Catalog #358004**

☒ Cell strainer 70um filter **Falcon Catalog #352350** .

☒ Percoll® **Sigma Aldrich Catalog #P4937** .

Microglia staining

Please refer staining protocol.

For culture experiments:

Use MG Cell Culture Media supplemented daily IL-34

A	B
Day0	40ng/mL IL34
After Day0	20ng/mL IL34

No media change necessary.

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Staining buffer:

A	B
HBSS	1X
BSA	1%
EDTA	1mM

A	B
HBSS	500 ml
BSA	5 g
EDTA	1 ml (0.5M EDTA stock)

Percoll
Isotonic Percoll

A	B
Percoll	45ml
10X HBSS	5 ml

e.g. 6 brains:

37% Percoll

A	B
IsoPercoll	13.32ml
1X HBSS	22.68 ml

70% Percoll

A	B
IsoPercoll	23.1 ml
1X HBSS	9.9 ml

MG Cell Culture Media

A	B
DMEM/F12	
FCS	5%
Anti-anti	

Microglia need approx. 6 days to recover in vitro after isolation before you start experiment.

Microglia isolation from mouse and culture (modified from UCSD) 1h 32m

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1 Deeply anaesthetize with CO_2.

1.1 Can also use euthanasia.

2 

Perfuse intracardially with ice-cold PBS (~10 to 20ml, liver gets white).

3 Remove whole brain and place into staining buffer (HBSS 1X – Life Technologies, 14175-095; 1% BSA, **1 millimolar (mM)** EDTA) **On ice** .

3.1 Place in 15 ml falcon tube.

4 Dissect brain into 6 pieces.

4.1 Place brain into mini cell culture dish with HBSS buffer.

4.2 Remove spinal cord completely.

4.3 Cut with dissecting blade.

- Remove cerebellum
- Cut forebrain into several pieces

4.4 Put brain pieces into 15ml falcon with HBSS.

5 

Gently homogenize in staining buffer (~ **10 mL**) using **2 mL** polytetrafluoroethylene pestle (Wheaton, 358026), for six brains (8-week-old) polypropylene round-bottom tube (Corning, 352059).

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5.1 Put brain into clear round bottom test tube (15 ml).

5.2 Gently go up and down with pestle a don't press down on bottom of tube.

5.3 Keep tubes **On ice** .

5.4 Homogenize for roughly **00:30:00** . 30m

6

When supernatant is cloudy, spin briefly to **48 g** , store supernatant in collection tube.

6.1 Acceleration=5

6.2 Deceleration=5

6.3

Wait until centrifuge gets to 48 and then immediately stop the spinning.

6.4 Put supernatant into a separate collection tube (label tube); 15 ml falcon tube.

6.5

Add HBSS + EDTA+BSA buffer into tube with brain chunks.

7 1. Then continue to homogenize the brain chunks in 15 ml tubes (Corning, 352063).

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7.1 Homogenize for roughly **00:20:00** . 20m

8 Spin briefly to **48 g** when supernatant is cloudy roughly **00:30:00** each time. 30m

8.1 Transfer supernatant to collection tube.

8.2 Put brain chunks into a smaller tube (corning 352063).

9 

Add buffer to brain chunks and repeat cycle until procedure becomes inefficient (You should have whitish stuff and supernatant is clear) Should have between 15 and 25 ml whitish to yellowish supernatant per brain.

9.1 Supernatant should become clear towards the end of dissociation (although there will still be white chunks of microglia left).

10 Lastly, transfer supernatant in 2 ml glass mortar (Wheaton, 358004), do once up & down with cell suspension SLOWLY.

10.1 Step is to remove and separate chunks of microglia.

10.2 

Add 1.6-1.8 of supernatant into glass mortar.

10.3 Use pestle to push down gently into liquid and then remove pestle and transfer supernatant to a new collection tube (50 ml falcon tube).

10.4 

Keep adding **2 mL** of the supernatant from the collection tube to the glass mortar and repeat this process for remaining supernatant.

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11 Filter homogenate onto 70 µm cell strainer (BD Falcon 352350).

11.1

Fill homogenate to **40 mL** with HBSS + EDTA +BSA in the 50 ml falcon tube.

11.2 Can switch to a new filter if it gets clogged.

12 12m

Centrifuge for **00:12:00** at **400 x g** at **18 °C** (step 5 acceleration, step 3 brakes).

12.1 Pour out supernatant.

13 Prepare Percoll (Sigma, P4937).

13.1

45 mL percoll + **5 mL** 10x HBSS (for 6 brains).

14

Resuspend pelleted homogenate in **6 mL** (per brain) of 37% isotonic Percoll in 15 ml centrifuge tube.

14.1 **37% percoll**

- 13.3 percoll (from step 13)
- 22.7 1x HBSS

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14.2 **70% percoll**

- 23.1 percoll (from step 13)
- 9.9 1x HBSS

15 

Underlay with **5 mL** of 70% isotonic Percoll.

*can also use **50 mL** Falcon, e.g. when 5 brains pooled. **22 mL** 37% isopercoll, underlay with **13 mL** 70% isopercol).

15.1 Use a 5 mL pipette for underlay.

15.2 Move pipette up very slow as you add more volume but make sure you are not too close to interphase.

16 

40m

Centrifuge at **600 x g** for **00:40:00** at **16 °C** - **18 °C** , with "0" acceleration and deceleration (!).

17 

Remove myelin and everything to about **3 mL** above the interphase.

17.1 

Remove 1-2 ml of debris on top.

18 Recover cells at the 37%–70% Percoll interphase, this should be ~2.5-3 ml.

18.1 

First put **2 mL** of HBSS+EDTA+BSA into a collection tube (15 ml falcon tube).

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18.2 Then get interphase (circle around with pipette).

18.3 Then get 2-3 ml above the interphase (don't collect liquid below interphase!).

18.4 

Remove **2 mL** of this separated cells into another tube for unstained control cells for FACS.

19  10m

Centrifuge for **00:10:00** at **400 x g** in HBSS 1X/MG media (1:1), remove supernatant.

19.1 Fill tube with HBSS before spinning.

20 

Repeat in **14 mL** Falcon tube in HBSS once or twice.

21 

Resuspend in **300 µL** of HBSS +EDTA + BSA.

22 Store cells in fridge for staining.

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