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Brain infiltrating leukocytes (BILs) extraction

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ASAP Collaborative Rese…

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We use this protocol and it's working

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Abstract

This protocol details the extraction of brain infiltrating leukocytes (BILs).

Materials

Reagents:

- RPMI 1640 Medium, GlutaMAX™ Supplement **Thermo Fisher Catalog #**61870036
- Liberase™ TL **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**05401020001
- DNAase I (Roche, Cat#04416728001)
- $CaCl_2$
- 090-150- FBS, Premium Canadian Origin, 500mL **Wisent Bioproducts Catalog #**090-150 (Heat inactivated 56 °C - 00:30:00)
- UltraPure 0.5M EDTA, pH 8.0 **Thermo Fisher Scientific Catalog #**15575-038
- Percoll® **Cytiva Catalog #**17-0891-02
- 811-410 FL- DPBS, 10X, with Calcium & Magnesium, 4L Not Sterile **Wisent Bioproducts Catalog #**811-410 FL
- Trypan Blue

Solutions:

1) Ammonium Chloride Potassium Red Cell Lysis buffer :

	A	B
	NH4Cl	8.29g
	KHCO3	1g
	EDTA	0.0367g
	distilled water	to 1L

2) CD45-FITC IP solution (25 µg/ml 200 µL **per mouse)**

CD45-FITC 50 µL of 0.5 mg/ml

950 µL PBS

3) Digestion enzyme buffer (1 mL **of 1x enzymes, per brain prepared right before the digestion)**

- RPMI No FBS + Liberase TL to 10 µg/ml (4 µl of stock Liberase TL 2.5 mg/ml per 1 ml of RPMI)
- DNAase to 40 µg/ml (4 µl of stock 10 mg/ml per 1 ml of Digestion buffer)
- Ca Cl_2 1.5-3 mM (of 100 mM stock take 1.5 µl per 1 ml of digestion buffer)

Note

! Liberase cannot be freeze-thawed.

4) FACS buffer (5 mL)

2% FBS (10 mL) in PBS 1 mM EDTA (1 mL of stock 0.5 M EDTA)

5) Gradient solution - 37% Percoll solution (10 mL per brain)

3.7 mL Percoll with 6.3 mL HBSS Room temperature (HBSS with Ca_2+ or D-PBS with Ca_2+)

Note

!! Needs Calcium

Plastics:

1) Cut 1 ml pipette tips (2-3 per brain)
2) 15ml conical tubes (per brain 1 -collection; 1- gradient centrifugation)
3) 50 ml conical tubes (per brain 1- for meshing through the strainer; 1- after gradient centrifugation)
4) 70 µm cell strainers (1 per brain)
5) Small petri dishes (1 per brain)
6) 3 ml syringes (plungers 1 per brain)
7) Black PP 96 well conical plates for FACS staining.
8) Scissors
9) Spatula
10) 1ml Insulin syringes with 27 G needles (1 per 5 mice)
11) Hemocytometer

Procedure (work as fast and as gentle as possible): 20m

1 Prior to sacking the mice, prepare 15 mL conical tubes with 3 mL RPMI and weigh each tube.

2 Inject mice with 200 µL of aCD45-FITC solution IP.

3 Sac mice exactly at 00:20:00 post injection by cervical dislocation. 20m

4 Carefully collect the brain with spatula to the 15 ml conical tube containing 3 ml RPMI and place On ice .

5 Weigh tube to calculate the weight of the brains.

6 Add brain into the petri dish, cut in small pieces with scissors.

7 Collect the brain pieces back to the collection 15 ml tube using a cut 1mL tip.

8 Spin down the contents at 1500 rpm, 4°C, 00:05:00 . While spinning prepare Digestion enzyme mix. 5m

9 Add 3 mL of RT digestion enzyme mix (1x) to each tube, mix gently (can be done using the vortex).

10 Incubate the tubes at 37 °C 00:35:00 with gentle shaking at 00:15:00 and 00:30:00 . 1h 20m

11 After the digestion, put the tube immediately On ice . Add 5 mL of PBS (with 2mM EDTA and 10% FBS) to neutralize the enzymes.

12 Prime cell strainers on top of the 50 ml tube with 10 mL FACS buffer.

13 Add the digested brain through the strainer. Add another 5 mL FACS to flush the strainer.

14 Add digested brain and push it with 3ml syringe plunger through the strainer, keep pouring FACS buffer to flush the strainer and the tube for digestion (up to 50mL).

15 Spin cells at 300 x g, Room temperature, 00:05:00 . 5m

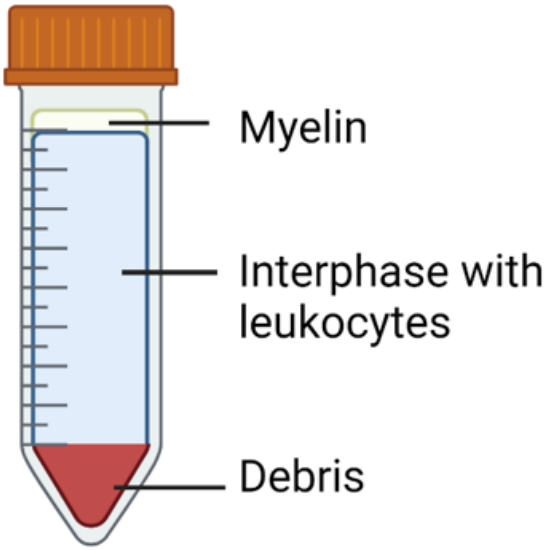
16 Prepare RT gradient (For each brain prepare 3.7 mL **of Percoll and** 5.3 mL **D-PBS** containing calcium and magnesium).

17 Discard supernatant after centrifugation and resuspend very gently the cell pellet in 1 mL **of D-PBS**. Transfer the pellet to the 15ml conical tube containing 9 mL of gradient solution. (Total gradient solution = 10mL).

18 Centrifuge at 500 x g, Room temperature, 00:20:00 **No Brake.** 20m

> **Note**
>
> It is essential that this step is done at RT. Make sure the centrifuge is set to RT early.

19 Collect and add the upper laye of myelin, using the cut 1 ml tips.



20 Collect the interphase up to the lowest red cell containing sediment and transfer it to the 50 ml tube (around 7mL).

21 Add ice cold PBS to the top and spin down at 400 x g, 4°C, 00:05:00 **with brake**. Discard the supernatant.

5m

22 **If any of the pellets are contaminated with RBC/debris then:**

5m 30s

Add 1 mL ACK lysis buffer and mix with the pipette and leave for for 00:00:30 and block the buffer with 4 mL of 10% FBS in PBS. Spin down at 400 x g, 4°C, 00:05:00 **with brake**. Discard the supernatant.

23 Resuspend the pellet in 200 µL of FACS buffer, take a small aliquot to stain trypan blue and count (from a UI mouse you expect to get 0.5-1x10^6 cells).

24 Adjust cell number and proceed to FACS staining.