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Protocol status: Working
We use this protocol and it's working

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Isolation of mouse brain pericytes (PDGFR-B+) using magnetic sorting (MACS)

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ABSTRACT

This protocol employs a modified procedure of the Adult Brain Dissociation kit (Miltenyi Biotec) to sort PDGFR-B+ cells from mouse brains. The protocol is adapted to enhance cell recovery and survival. The original protocol can be seen in:

https://www.miltenyibiotec.com/CA-en/products/adult-brain-dissociation-kit-mouse-and-rat.html#130-107-677

GUIDELINES

This protocol makes use of the reagents contained in the **Adult Brain Dissociation Kit, mouse and rat (**Miltenyi biotec, cat# 130-107-677).

MATERIALS

This protocol makes use of the reagents contained in the **Adult Brain Dissociation Kit, mouse and rat (**Miltenyi biotec, cat# 130-107-677).

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PROTOCOL MATERIALS

PBS - Phosphate-Buffered Saline (10X) pH 7.4 **Thermo Fisher Scientific Catalog #AM9625**

Step 2

Debris Removal Solution **Miltenyi Biotec Catalog #130-109-398** Step 10

Red Blood Cell Lysis Solution (10×) **Miltenyi Biotec Catalog #130-094-183**

In 2 steps

BD Pharmingen™ Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™) **BD Biosciences Catalog #553142**

Step 18

LIVE/DEAD™ Fixable Blue **Thermo Fisher Scientific Catalog #L23105** In 2 steps

UltraComp eBeads **Thermo Fisher Scientific Catalog #01-2222-42** Step 20

CD140b Antibody **Miltenyi Biotec Catalog #130-123-271** Step 19

BEFORE START INSTRUCTIONS

Read the full protocol

Mouse euthanasia and tissue dissection

40m

1 Euthanize mice employing CO2 or decapitation according to institutional guidelines.

2 Remove the brain following standard procedures, avoiding tissue damage. Place the brain in falcon tubes or 10-cm Petri dishes filled with 1x

PBS - Phosphate-Buffered Saline (10X) pH 7.4 **Thermo Fisher Scientific Catalog #AM9625**

On ice .

3 Prepare the enzyme mix 1 and 2 according to the table (quantities per brain)

	A	B	C	D
	Mix 1		Mix 2	
	Enzyme P	Buffer Z	Buffer Y	Enzyme A

	A	B	C	D
	50 ul	1900 ul	20 ul	10 ul

Step 3 includes a Step case.
Using gentleMAC Octo Dissociator
Using rotor or hybridization chamber

step case

Using gentleMAC Octo Dissociator

Device: Cat# 130-096-427
Tubes: Cat # 130-093-237

Transfer the brains to a 10-cm Petri dish. Use forceps and a razor blade/surgical scalpel to mince the tissue into small pieces (approx 1 mm x 1mm).

4 Place the pieces into the tubes filled with Mix1 and Mix2. **Close the cap tightly** to prevent liquid leakage.

Mouse euthanasia and tissue dissection

40m

5 Place the C tubes into the **octo dissociator** with heaters and execute the program **37C_ABDK_1**

Cell dissociation and enzymatic digestion

35m

6 When the digestion is complete, resuspend the samples and add them into a **moisten** 70 µL cell filter attached to a 50 mL falcon tube. Do not discard 15 mL tubes at this point.

7 Add 7 mL of cold **D-PBS** to the falcon tubes used for digestion. Mix briefly to recover any sample left. Pass the buffer through the filter using a glass pipette. Discard the filter and the 15 mL falcon tubes.

8 Divide the sample in 2 15 mL falcon tubes.

Note

This step favors the coming debris removal. In our experience, the division of a single hemisphere into two samples gives optimal results.

9 Centrifugate the samples at 300 x g, 4°C, 00:05:00 5m

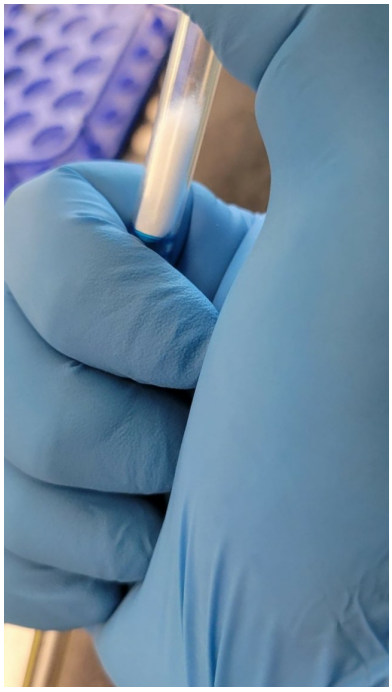
Debris removal 15m

10 Decant the supernadant and resuspend the pellet in 3100 µL of **D-PBS +** 900 µL Debris Removal Solution **Miltenyi Biotec Catalog #130-109-398** .

11 In this step, a gradient is performed. Overlay very gently 4 mL of cold **D-PBS using a glass pipet.** In the end, **two phases** must be easily recognizable. .

Note

We recommend doing this manually, controlling the PBS fluid with the thumb



12 Centrifuge 3000 x g, 4°C, 00:10:00 with **reduced acceleration and brake** 10m

Acceleration: 1
Brake: 1

Note

If the acceleration and brake are at full speed, the debris removal is sub-optimal.

13 Aspirate the first two phases and fill up the tube **up to** 5 mL with **PBS**. Gently invert the tube three times.

14 Centrifugate 1000 x g, 4°C, 00:10:00 and decant supernadant. 10m

Red Blood Cell Removal (Optional step) 15m

15 Dilute the Red Blood Cell Lysis Solution (10×) **Miltenyi Biotec Catalog #130-094-183** at 1:10 using **double-distilled water (ddH2O)**.

Note

IMPORTANT: Use **only ddH2O**, not distilled water or other buffers.

16 Resuspend the pellet in 1 mL of Red Blood Cells Removal Solution, **transfer to FACS tubes,** and incubate for 00:10:00 in the refrigerator 4 °C 10m

Note

If the samples have been divided before debris removal, they can be merged again in this step. Each sample can be homogenized in 500 µL of Red Blood Cell Lysis Solution (10×) **Miltenyi Biotec Catalog #130-094-183** and then merged in a single FACS tube.

17 When incubation is finished, add 4 mL of cold **HBSS/BSA/Glucose buffer,** and centrifuge 300 x g, 4°C, 00:05:00 . Aspirate the supernatant afterwards. 5m

Cell staining 35m

18 Resuspend the cells in 100 µL of **HBSS/BSA/Glucose buffer** and add 1 µL of

BD Pharmingen™ Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™) **BD Biosciences Catalog #553142**

. Incubate for 10 minutes at 4 °C .

19 30m

Add 10 µL of CD140b Antibody **Miltenyi Biotec Catalog #130-123-271** (1:10 dilution) and 1 µL of LIVE/DEAD™ Fixable Blue **Thermo Fisher Scientific Catalog #L23105** (1:100 dilution) to label dead cells.
Incubate for 00:30:00 at 4 °C in the dark.

Note

These steps involving control sample preparation are **only suitable if subsequent FACS is intended**. For MACS (only) experiments, incubation of the experimental samples with the blocking solution and desired antibody is sufficient. **Go directly to step 22.**

If additional stainings are performed, add the required antibody amount according to titration and set corresponding controls = Single-color positive controls and fluorescence minus one (FMO) controls.

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20

At this point, the researcher should also set the staining controls for the experiment. For this simple setup, prepare:

1) A sample containing 100-200 µL of unstained cells (Control is ready for FACS)
2) A FACS tube containing 1 drop of UltraComp eBeads **Thermo Fisher Scientific Catalog #01-2222-42** 1 µL of antibody, and 100 µL of **HBSS/BSA/Glucose buffer (**Control readyACS)
3) A FACS tube containing one drop of ArC reactive beads (green cap), 1 µL of LIVE/DEAD™ Fixable Blue **Thermo Fisher Scientific Catalog #L23105** (1:100 dilution).

21 30m

Incubate the third control sample (together with the experimental samples) for 00:30:00 **.**

22 When antibody incubation is finished, add 2 mL of **HBSS/BSA/Glucose buffer** to the samples and control number 3 and centrifuge 300 x g, 4°C, 00:05:00 5m

23 Decant the supernatant. In control sample 3, add 1 drop of ArC negative beads (white cap) and 100 µL of **HBSS/BSA/Glucose buffer.** This control sample is ready for FACS.

Otherwise, experimental samples must be resuspended in 80 µL of **HBSS/BSA/Glucose buffer** to perform magnetic separation (MACS sorting).

24 Add 20 µL of **magnetic beats** according to your antibody incubation (Anti-PE magnetic beats or anti-APC magnetic beats) and incubate for 00:15:00 at 4 °C . 15m

25 Prepare and label FACS or Eppendorf tubes to collect the negative fraction (140b negative cells), and positive fractions (140b positive cells).

26 When incubation with the magnetic beats is finished, add 2 mL of **HBSS/BSA/Glucose buffer** and 300 x g, 4°C, 00:10:00 . Discard the supernatant, and resuspend the cells in 500 µL of **PBS + 2% FCS** 10m

Note

PBS is recommended at this step to avoid excessive bubbling during MACS.

Magnetic Separation (MACS sorting) 5m

27 Place the magnetic columns in a suitable MACS separator and rinse each column (MS columns) with 500 µL of **PBS + 2% FCS** until the buffer is decanted entirely by gravity.

Note

Place a container below the magnet to collect buffer remains.

28 Using a FACS tube rack, match negative fractions FACS (or Eppendorf) tubes to each column and apply the 500 µL of cell suspension into each column. When the buffer reservoir is empty, add 500 µL of **PBS + 2% FCS** three times.

29 When all the negative fraction is collected, **remove the columns from the magnet** and place it on FACS or Eppendorf tubes intended to collect the **140b positive cells**. Pipette 1 mL of **PBS + 2% FCS** into the columns and firmly push the plunger into the column.

In the end, the research has a tube with a negative fraction (all cells not PDGFR-B+) and a positive fraction (PDGFR-B+ labeled with magnetic beats).

Note

To increase the purity of 140b positive cells, the previous step can be repeated using a new magnetic collum.

30 Centrifugate the samples 300 x g, 4°C, 00:05:00 and resuspend in 300 µL of **PBS + 2% FCS.** Samples are ready for FACS. 5m

If FACS is not performed, aspirate the supernatant and freeze the cells, or place Othem in an appropriate buffer for subsequent experimental procedures.

Note

Depending on the experimental porpuses, the experimenter can discard the negative fractions collected during magnetic separation.

Consider that the positive fraction gives strong interference bands at the level of 60 Kda in **western blots** given the presence of beats.