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

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Protocol status: Working

We use this protocol and it's working

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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).

Protocol materials

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

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

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

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

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

☒ LIVE/DEAD™ Fixable Blue **Thermo Fisher Scientific Catalog #L23105**

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

☒ CD140b Antibody **Miltenyi Biotec Catalog #130-123-271**

☒ LIVE/DEAD™ Fixable Blue **Thermo Fisher Scientific Catalog #L23105**

Troubleshooting

Before start


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
	50 ul	1900 ul	20 ul	10 ul

STEP CASE

Using gentleMAC Octo Dissociator 27 steps

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



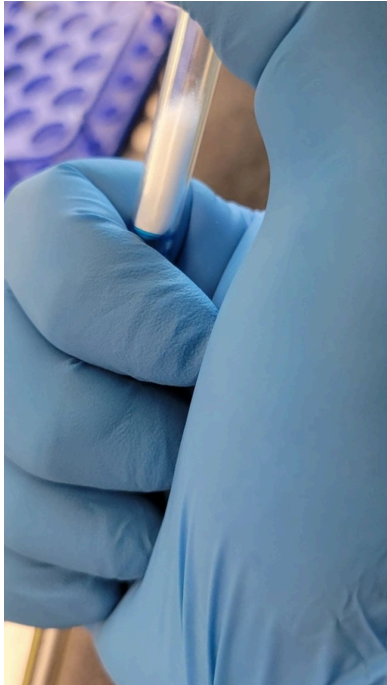
Debris removal


15m

- 10 Decant the supernatant 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 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.

- 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 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


30m


30m




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 beads** according to your antibody incubation (Anti-PE magnetic beads or anti-APC magnetic beads) 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 beads 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 μ L 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 beads).

Note

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

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

5m



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

Note

Depending on the experimental purposes, 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 beads.

Protocol references

https://static.miltenyibiotec.com/asset/150655405641/document_nk45qkndm94opfhalsuup3b909?content-disposition=inline

<https://www.nature.com/articles/nprot.2017.158>