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### 1. Description

<b>Components</b>	2 mL Anti-Prominin-1 MicroBeads, mouse: MicroBeads conjugated to monoclonal anti-mouse Prominin-1 antibodies (isotype: rat IgG1).
<b>Capacity</b>	For 10 <sup>9</sup> total cells, up to 100 separations.
<b>Product format</b>	Anti-Prominin-1 MicroBeads are supplied in buffer containing stabilizer and 0.05% sodium azide.
<b>Storage</b>	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

#### 1.1 Principle of the MACS® Separation

First, the prominin-1<sup>+</sup> cells are magnetically labeled with Anti-Prominin-1 MicroBeads. Then, the cell suspension is loaded onto a MACS® Column, which is placed in the magnetic field of a MACS Separator. The magnetically labeled prominin-1<sup>+</sup> cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of prominin-1<sup>+</sup> cells. After removing the column from the magnetic field, the magnetically retained prominin-1<sup>+</sup> cells can be eluted as the positively selected cell fraction. To increase the purity, the positively selected cell fraction containing the prominin-1<sup>+</sup> cells is separated over a second column.

#### 1.2 Background information

Anti-Prominin-1 MicroBeads have been developed for the separation of mouse cells based on the expression of prominin-1. Prominin-1 is a 115-120 kDa, 5-transmembrane glycoprotein with close structural similarity to its human ortholog, CD133. Prominin-1 is expressed on neural stem cells, for example in the white matter of the cerebellum<sup>1</sup>, neuroepithelial tissue and various other epithelia in the mouse embryo<sup>2</sup>. Five to ten percent of the neural cells found in the subventricular zone of postnatal mice were shown to be

prominin-1<sup>+</sup>. In the adult mouse, prominin-1 has been detected in the brain ependymal layer, and in kidney tubules.<sup>2</sup>

#### 1.3 Applications

- Positive selection or depletion of cells expressing mouse prominin-1.
- Isolation of prominin-1<sup>+</sup> neural stem cells from mouse cerebellar tissue, firstly by lineage depletion of neuronal and glial progenitor cells using CD81 (TAPA-1), PSA-NCAM and O4 followed by positive selection for prominin-1<sup>+</sup> cells in the flow through.

#### 1.4 Reagent and instrument requirements

- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 with autoMACS™ Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C). Degas buffer before use, as air bubbles could block the column.

▲ **Note:** EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). BSA can be replaced by other proteins such as mouse serum albumin, mouse serum, or fetal bovine serum (FBS). Buffers or media containing Ca<sup>2+</sup> or Mg<sup>2+</sup> are not recommended for use.

- MACS Columns and MACS Separators: Prominin-1<sup>+</sup> cells can be enriched by using MS, LS, or XS Columns or depleted with the use of LD, CS, or D Columns. Cells which strongly express prominin-1 can also be depleted using MS, LS, or XS Columns. Positive selection or depletion can also be performed by using the autoMACS Pro or the autoMACS Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
<b>Positive selection</b>			
MS	10 <sup>7</sup>	2×10 <sup>8</sup>	MiniMACS, OctoMACS, VarioMACS, SuperMACS
LS	10 <sup>8</sup>	2×10 <sup>9</sup>	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
XS	10 <sup>9</sup>	2×10 <sup>10</sup>	SuperMACS
<b>Depletion</b>			
LD	10 <sup>8</sup>	5×10 <sup>8</sup>	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
CS	2×10 <sup>8</sup>		VarioMACS, SuperMACS
D	10 <sup>9</sup>		SuperMACS
<b>Positive selection or depletion</b>			
autoMACS	2×10 <sup>8</sup>	4×10 <sup>9</sup>	autoMACS Pro, autoMACS

▲ **Note:** Column adapters are required to insert certain columns into the VarioMACS™ or SuperMACS™ Separators. For details see the respective MACS Separator data sheet.

- Neural Tissue Dissociation Kit (P) (# 130-092-628) for the generation of single-cell suspensions of neural cells from mouse brain tissue.

- gentleMACS™ Dissociator (# 130-092-235).
- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., Anti-Prominin-1-PE (# 130-092-334) or Anti-Prominin-1-APC (# 130-092-335). For more information about fluorochrome-conjugated antibodies see [www.miltenyibiotec.com](http://www.miltenyibiotec.com).
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells.
- (Optional) Dead Cell Removal Kit (# 130-090-101) for the depletion of dead cells.
- (Optional) Pre-Separation Filters (# 130-041-407) to remove cell clumps.

## 2. Protocol

### 2.1 Sample preparation

When working with tissues or lysed blood, prepare a single-cell suspension using standard methods. For preparing a single-cell suspension please refer to our gentleMACS™ Protocol “Preparation of single-cell suspension from mouse neural tissue”.

For details see the protocols section at [www.miltenyibiotec.com/protocols](http://www.miltenyibiotec.com/protocols).

▲ For the preparation of cells from neural tissue refer to the data sheet of the Neural Tissue Dissociation Kit (P) (# 130-092-628), which can be used in combination with the gentleMACS™ Dissociator (# 130-093-235).

▲ Dead cells may bind non-specifically to MACS MicroBeads. To remove dead cells, we recommend using density gradient centrifugation or the Dead Cell Removal Kit (# 130-090-101).



### 2.2 Magnetic labeling

▲ Work fast, keep cells cold, and use pre-cooled solutions. This will prevent capping of antibodies on the cell surface and non-specific cell labeling.

▲ Volumes for magnetic labeling given below are for up to  $10^7$  total cells. When working with fewer than  $10^7$  cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for  $2 \times 10^7$  total cells, use twice the volume of all indicated reagent volumes and total volumes).

▲ For optimal performance it is important to obtain a single-cell suspension before magnetic labeling. Pass cells through 30  $\mu$ m nylon mesh (Pre-Separation Filters, # 130-041-407) to remove cell clumps which may clog the column. Moisten filter with buffer before use.

▲ The recommended incubation temperature is 2–8 °C. Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

1. Determine cell number.
2. Centrifuge cell suspension at  $300 \times g$  for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 80  $\mu$ L of buffer per  $10^7$  total cells.
4. Add 20  $\mu$ L of Anti-Prominin-1 MicroBeads per  $10^7$  total cells.
5. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).

6. (Optional) Add staining antibodies, e.g., 10  $\mu$ L of Anti-Prominin-1-APC (# 130-092-335), and incubate for 5 minutes in the dark in the refrigerator (2–8 °C).
7. Wash cells by adding 1–2 mL of buffer per  $10^7$  cells and centrifuge at  $300 \times g$  for 10 minutes. Aspirate supernatant completely.
8. Resuspend up to  $10^8$  cells in 500  $\mu$ L of buffer.
  - ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
  - ▲ **Note:** For depletion with LD Columns, resuspend up to  $1.25 \times 10^8$  cells in 500  $\mu$ L of buffer.
9. Proceed to magnetic separation (2.3).



### 2.3 Magnetic separation

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of prominin-1<sup>+</sup> cells. For details see table in section 1.4.

▲ Always wait until the column reservoir is empty before proceeding to the next step.

▲ To achieve highest purities, purification of prominin-1<sup>+</sup> cells should be performed with two consecutive column runs.

#### Magnetic separation with MS or LS Columns

1. Place column in the magnetic field of a suitable MACS Separator. For details see the respective MACS Column data sheet.
2. Prepare column by rinsing with the appropriate amount of buffer:
 

MS: 500 $\mu$ L	LS: 3 mL
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3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.
4. Wash column with the appropriate amount of buffer. Collect unlabeled cells that pass through and combine with the effluent from step 3.

MS: $3 \times 500 \mu$ L	LS: $3 \times 3$ mL
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▲ **Note:** Perform washing steps by adding buffer aliquots only when the column reservoir is empty.

5. Remove column from the separator and place it on a suitable collection tube.
6. Pipette the appropriate amount of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.

MS: 1 mL	LS: 5 mL
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7. (Optional) To increase the purity of prominin-1<sup>+</sup> cells, the eluted fraction can be enriched over a second MS or LS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

▲ **Note:** Elution of the cells from the column after the second separation should be performed with cell culture medium if cells are to be taken directly into culture, otherwise elute with buffer as before.

▲ **Note:** Keep handling times of cells in PBS/EDTA/BSA buffer to a minimum. Cells must only be stored in cell culture medium after enrichment over the columns in order to preserve cell viability.

#### Magnetic separation with XS Columns

For instructions on the column assembly and the separation refer to the XS Column data sheet.

### Depletion with LD Columns

1. Place LD Column in the magnetic field of a suitable MACS Separator. For details see LD Column data sheet.
2. Prepare column by rinsing with 2 mL of buffer.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with 2×1 mL of buffer. Collect total effluent; this is the unlabeled cell fraction. Perform washing steps by adding buffer two times. Only add new buffer when the column reservoir is empty.

### Depletion with CS Columns

1. Assemble CS Column and place it in the magnetic field of a suitable MACS Separator. For details see CS Column data sheet.
2. Prepare column by filling and rinsing with 60 mL of buffer. Attach a 22G flow resistor to the 3-way stopcock of the assembled column. For details see CS Column data sheet.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with 30 mL buffer from the top. Collect total effluent; this is the unlabeled cell fraction.

### Depletion with D Columns

For instructions on column assembly and separation refer to the D Column data sheet.

### Magnetic separation with the autoMACS™ Pro Separator or the autoMACS™ Separator

- ▲ Refer to the respective user manual for instructions on how to use the autoMACS™ Pro Separator or the autoMACS Separator.
- ▲ Buffers used for operating the autoMACS Pro Separator or the autoMACS Separator should have a temperature of  $\geq 10$  °C.
- ▲ Program choice depends on the isolation strategy, the strength of magnetic labeling, and the frequency of magnetically labeled cells. For details refer to the section describing the cell separation programs in the respective user manual.

#### Magnetic separation with the autoMACS™ Pro Separator

1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and the fraction collection tubes in rows B and C.
3. For a standard separation choose one of the following programs:  
Positive selection: "Posseld2"  
Collect positive fraction in row C of the tube rack.  
Depletion: "Depletes"  
Collect negative fraction in row B of the tube rack.

#### Magnetic separation with the autoMACS™ Separator

1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube at the uptake port and the fraction collection tubes at port neg1 and port pos2.

3. For a standard separation choose one of the following programs:

Positive selection: "Posseld2"

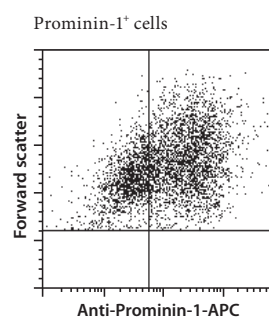
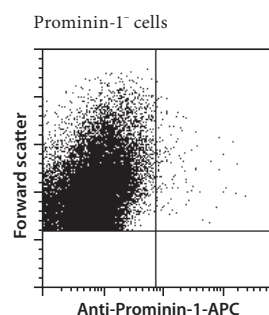
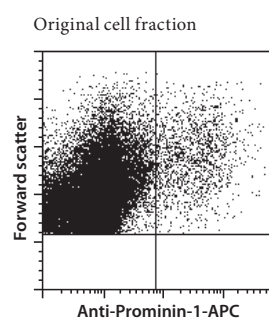
Collect positive fraction from outlet port pos2.

Depletion: "Depletes"

Collect negative fraction from outlet port neg1.

### 3. Example of a separation using the Anti-Prominin-1 MicroBeads

Separation of a single-cell suspension derived from day 1 postnatal mouse cerebellar tissue using the Neural Tissue Dissociation Kit (P), Anti-Prominin-1 MicroBeads, two MS Columns, and a MiniMACS™ Separator. Cells were fluorescently stained with Anti-Prominin-1-APC (# 130-092-335) and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



### 4. References

1. Lee, A. *et al.* (2005) Isolation of neural stem cells from the postnatal cerebellum. *Nat. Neurosci.* 8: 723–729.
2. Weigmann, A. *et al.* (1997) Prominin, a novel microvilli-specific polytopic membrane protein of the apical surface of epithelial cells, is targeted to plasmalemmal protrusions of non-epithelial cells. *Proc. Natl. Acad. Sci, USA* 94: 12425–12430.

All protocols and data sheets are available at [www.miltenyibiotec.com](http://www.miltenyibiotec.com).

## 5. Appendix: Dissociation of mouse brain tissue using the Neural Tissue Dissociation Kit (P)

### Reagent and instrument requirements

- Neural Tissue Dissociation Kit (P) (# 130-092-628)
- HBSS w/o Ca<sup>2+</sup> or Mg<sup>2+</sup> (Sigma-Aldrich # H4891)
- beta-mercaptoethanol (e.g. Sigma # 63689)
- 30 µm cell strainer (e.g. Pre-Separation Filters (# 130-041-407)
- Sterile scalpel
- 35 mm diameter sterile petri dish
- Sterile glass pipettes
- (Optional) Sterile filters (e.g. Millipore # SLGP R25 LS)
- (Optional) 0.9 M sucrose solution in HBSS, pH 7–7.5
- MACSmix™ Tube Rotator (# 130-090-753) or water bath at 37 °C

### Reagent and instrument preparation

▲ For cell culture experiments subsequent to tissue dissociation, Solution 4 of the Neural Tissue Dissociation Kit (P) should be sterile filtered.

▲ Volumes given below are for up to 400 mg of starting tissue material. When working with less than 400 mg, use the same volumes as indicated. When working with more than 400 mg, scale up all reagent volumes and total volumes accordingly. Tissue quantities of 400 mg and less can be processed in a single 2 mL reaction tube, while tissue quantities of greater than 400 mg can be pooled and processed in an appropriate-sized conical tube.

▲ **Note:** The weight of a mouse brain (C57/BL6, postnatal day 6) corresponds to approximately 200 mg).

1. Add beta-mercaptoethanol to Solution 2 to a final concentration of 0.067 mM. For example, add 13.5 µL of 50 mM beta-mercaptoethanol to 10 mL of Solution 2.  
▲ **Note:** This solution will then be stable for 1 month at 4 °C.
2. Resuspend the lyophilized powder in the vial labeled Solution 4 with 1 mL of Storage Buffer for Solution 4. Do **not** vortex. This solution should then be sterile filtered in the case of cell culture applications, aliquoted and stored at –20 °C for later use.

Table 1:

Enzyme mix 1		Enzyme mix 2	
Solution 1	Solution 2	Solution 3	Solution 4
50 µL	1900 µL	20 µL	10 µL

### Neural tissue dissociation protocol

▲ For cell culture experiments subsequent to tissue dissociation, all steps should be performed under sterile conditions.

▲ This protocol describes the dissociation of mouse brain tissue, though, in principle, it is transferable to other neural tissue types.

1. Fire polish three glass pipettes so that decreasing tip diameters are achieved.
2. Prepare 1950 µL enzyme mix 1 per 400 mg tissue by adding 50 µL of Solution 1 to 1900 µL of Solution 2 (see table 1) and vortex. Preheat the mixture at 37 °C for 10–20 min before use.
3. Remove the mouse brain. Determine the weight of tissue in 1 mL of cold HBSS to make sure the 400 mg limit per digestion is not exceeded.
4. Place the brain on the lid of a 35 mm diameter petri dish, remove the meninges (optional), and cut brain into small pieces using a scalpel.

▲ **Note:** For certain applications such as cultivation of neuronal cells, meninges should be removed.

5. Using a 1 mL pipette tip, add 1 mL of cold HBSS and pipette pieces back into an appropriate-sized tube (see below). Rinse with HBSS.  
▲ **Note:** When using <200 mg of brain tissue, return pieces to a 2 mL reaction tube. For tissue quantities >200 mg, pipette pieces into a 15 mL conical tube.  
▲ **Note:** When working with mice >P10, cut 2 mm off the end of the pipette tip to facilitate pipetting.
6. Centrifuge at 300 ×g for 2 min at 4 °C and aspirate the supernatant carefully.
7. Add 1950 µL of pre-heated enzyme mix 1 (Solutions 1 and 2) per up to 400 mg tissue. Incubate in closed tubes for 20 minutes at 37 °C under slow, continuous rotation using a MACSmix Tube Rotator. Alternatively incubate in a water bath, inverting the tube several times every 5 minutes to resuspend settled cells.
8. Prepare 30 µL enzyme mix 2 per 400 mg tissue by adding 20 µL of Solution 3 to 10 µL of Solution 4 (see table 1). Then add to sample.
9. Invert gently to mix. Do **not** vortex.
10. Dissociate tissue mechanically using the wide-tipped, fire-polished Pasteur pipette by pipetting up and down 10 times slowly. Avoid forming air bubbles.
11. Incubate at 37 °C for 15 min using a MACSmix Tube Rotator or in a water bath, agitating as above.
12. Dissociate tissue mechanically using the other two fire-polished pipettes in decreasing diameter. Pipette slowly up and down 10 times with each, or as long as tissue pieces are still observable. Care to avoid the formation of air bubbles.
13. Apply the single-cell suspension to a 30 µm cell strainer and wash with 10 mL of HBSS. Discard cell strainer and centrifuge at 300 ×g for 10 min at 4 °C.  
▲ **Note:** Cells with a diameter >30 µm, such as Purkinje cells or motor neurons, may be lost. To obtain these cells within the flow through, use a cell strainer with an appropriate diameter.
14. Repeat washing and centrifugation step.  
▲ **Note:** If problems with the formation of a compact pellet occur after either washing step, add 20 µL of Solution 3 and 10 µL of Solution 4 per mL of cell suspension, mix gently and incubate for a minimum of 5 minutes at 37 °C in a water bath, followed by washing as above.
15. (Optional, instead of step 14) For myelin removal, discard supernatant and resuspend cell pellet in 10 mL of cold sucrose solution. Centrifuge at 850 ×g, 4 °C for 10 min, then discard the supernatant carefully. Finally, resuspend in 10 mL of HBSS and centrifuge at 300 ×g for 10 min at 4 °C.  
▲ **Note:** Though removal of myelin will increase purities during subsequent flow cytometric analyses and cell separation using MACS Technology, the overall yield of cells will decrease.
16. Cells should be processed immediately for further applications.

### Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

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