Miltenyi Biotec

Anti-Prominin-1 MicroBeads

mouse

1 mL	130-092-333
100 µL	130-092-564

Contents

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.

1. Description

This product is for research use only.

Components	**1 mL Anti-Prominin-1 MicroBeads, mouse** or **100 µL Anti-Prominin-1 MicroBeads, mouse – small size:** MicroBeads conjugated to monoclonal anti-mouse Prominin-1 antibodies (rat IgG1).
Capacity	1 mL for 5×10^8 total cells, up to 50 separations or 100 µL for 5×10^7 total cells, up to 5 separations.
Product format	Anti-Prominin-1 MicroBeads are supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	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$^+$ 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$^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of prominin-1$^+$ cells. After removing the column from the magnetic field, the magnetically retained prominin-1$^+$ cells can be eluted as the positively selected cell fraction. To increase the purity, the positively selected cell fraction containing the prominin-1$^+$ 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[1], neuroepithelial tissue, and various other epithelia in the mouse embryo[2]. Five to ten percent of the neural cells found in the subventricular zone of postnatal mice were shown to be prominin-1$^+$. In the adult mouse, prominin-1 has been detected in the brain ependymal layer, and in kidney tubules.[2]

1.3 Applications

- Positive selection or depletion of cells expressing mouse prominin-1.
- Isolation of prominin-1$^+$ 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$^+$ cells in the flow through.

1.4 Reagent and instrument requirements

- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, and 0.5% bovine serum albumin (BSA) 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:** BSA can be replaced by other proteins such as appropriate serum albumin, appropriate serum, or fetal bovine serum (FBS). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- MACS Columns and MACS Separators: Prominin-1$^+$ cells can be enriched by using MS or LS Columns or depleted with the use of LD Columns. Cells which strongly express prominin-1 can also be depleted using MS or LS Columns. Positive selection or depletion can also be performed by using the autoMACS Pro Separator or the MultiMACS™ Cell24 Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Positive selection			
MS	10^7	2×10^7	MiniMACS, OctoMACS
LS	2×10^7	4×10^7	MidiMACS, QuadroMACS
Depletion			
LD	1.5×10^7	3×10^7	MidiMACS, QuadroMACS

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Positive selection or depletion

autoMACS	5×10^7	10^8	autoMACS Pro
Multi-24	2×10^7	4×10^7	MultiMACS Cell24

- 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), gentleMACS Octo Dissociator (# 130-095-937), or gentleMACS Octo Dissociator with Heaters (# 130-096-427).
- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., Prominin-1-PE or Prominin-1-APC. For more information about fluorochrome-conjugated antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells.
- (Optional) MACS SmartStrainers (30 µm) (# 130-098-458) to remove cell clumps.

2. Protocol

2.1 Sample preparation

For the preparation of single-cell suspensions from neural tissues 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 (#130-092-235).

For details refer to the protocols section at www.miltenyibiotec.com/protocols.

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 2×10^7 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 µm nylon mesh (MACS SmartStrainers (30 µm), # 130-098-458) to remove cell clumps which may clog the column. Moisten filter with buffer before use.

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

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 80 µL of buffer per 10^7 total cells.
4. Add 20 µL 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 µL of Prominin-1-APC, 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×g for 10 minutes. Aspirate supernatant completely.
8. Resuspend up to 10^8 cells in 500 µL of buffer.
   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
   ▲ **Note:** For depletion with LD Columns, resuspend up to 1.25×10^8 cells in 500 µL 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$^+$ cells. For details refer to 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$^+$ 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 refer to the respective MACS Column data sheet.
2. Prepare column by rinsing with the appropriate amount of buffer:
   MS: 500 µL LS: 3 mL
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: 3×500 µL LS: 3×3 mL
   ▲ **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
7. (Optional) To increase the purity of prominin-1$^+$ 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 cuture medium after enrichment over the columns in order to preserve cell viability.

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Depletion with LD Columns

1. Place LD Column in the magnetic field of a suitable MACS Separator. For details refer to the 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.

Magnetic separation with the MultiMACS™ Cell24 Separator

Refer to the the MultiMACS™ Cell Separator user manual for instructions on how to use the MultiMACS Cell24 Separator.

Magnetic separation with the autoMACS® Pro Separator

▲ Refer to the respective user manual for instructions on how to use the autoMACS® Pro Separator.

▲ Buffers used for operating the autoMACS Pro Separator should have a temperature of ≥ 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.

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

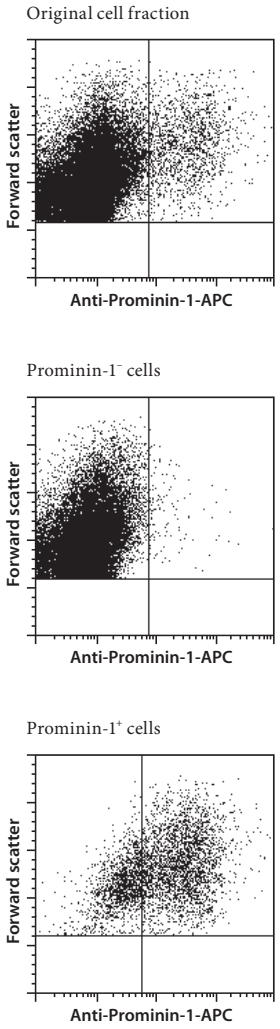
   Collect positive fraction in row C of the tube rack.

   Depletion: Depletes

   Collect negative fraction in row B of the tube rack.

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 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.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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