Miltenyi Biotec

Anti-PSA-NCAM MicroBeads

human, mouse, rat

1 mL	130-092-966
100 µL	130-092-981

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-PSA-NCAM MicroBeads, human, mouse, rat** or **100 µL Anti-PSA-NCAM MicroBeads, human, mouse, rat – small size:** MicroBeads conjugated to monoclonal anti-mouse PSA-NCAM antibodies (isotype: mouse IgM).
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-PSA-NCAM 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 MACS® Separation

First, the PSA-NCAM$^+$ cells are magnetically labeled with Anti-PSA-NCAM 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 PSA-NCAM$^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of PSA-NCAM$^+$ cells. After removing the column from the magnetic field, the magnetically retained PSA-NCAM$^+$ cells can be eluted as the positively selected cell fraction.

1.2 Background information

Anti-PSA-NCAM MicroBeads have been developed for the isolation of PSA-NCAM$^+$ cells. The Anti-PSA-NCAM antibody recognizes polysialic acid (PSA) which, in vertebrates, is linked to the extracellular domain of the neural cell adhesion molecule (NCAM, CD56)[1]. PSA-NCAM, the highly polysialated form of NCAM, is predominantly expressed in embryonic and neonatal neural tissue.[2] In adult mammalian brain PSA-NCAM expression is restricted mainly to areas that retain neurogenic potential, such as the subventricular zone (SVZ)[3] and the dentate gyrus of the hippocampus[4].

PSA-NCAM is a marker for immature neuronal-committed progenitors that are permanently generated in the SVZ and migrate along a well-defined pathway, the rostral migratory stream, into the olfactory bulb where they differentiate into GABAergic and dopaminergic interneurons.[3,5]

Antibodies against PSA-NCAM have been used to immunomagnetically isolate neuronal progenitors from postnatal mice and rat forebrain.[6–8]

1.3 Applications

- Positive selection or depletion of cells expressing PSA-NCAM.
- Positive selection of neuronal progenitor cells, e.g. from mouse SVZ tissue in conjunction with prior depletion of A2B5$^+$ glial progenitor cells.[6,7]

1.4 Reagent and instrument requirements

- Buffer: Prepare a solution containing phosphate-buffered saline (PBS) pH 7.2, 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 human serum albumin, human 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: PSA-NCAM$^+$ 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 the PSA-NCAM antigen can also be depleted using MS, LS, or XS 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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140-001-988.06

Positive selection or depletion

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

- (Optional) Neural Tissue Dissociation Kit (T) (# 130-093-231) for the generation of single-cell suspensions of neural cells from mouse and rat neural tissue.
- (Optional) gentleMACS™ Dissociator (# 130-093-235), gentleMACS Octo Dissociator (# 130-095-937), or gentleMACS Octo Dissociator with Heaters (# 130-096-427) and C Tubes (# 130-093-237, # 130-096-334)
- Pre-Separation Filters, 30 µm (# 130-041-407) to remove cell clumps.
- FcR Blocking Reagent, mouse (# 130-092-575) or human (# 130-059-901) to avoid Fc receptor-mediated antibody labeling.
- Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., PSA-NCAM-PE or PSA-NCAM-APC. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells.

2. Protocol

2.1 Sample preparation

Prepare a single-cell suspension from your tissue of interest by using the Neural Tissue Dissociation Kit (T) (# 130-093-231), which can also be used in combination with the gentleMACS Dissociators. For details please refer to the respective data sheets.

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 separation. Pass cells through 30 µm nylon mesh (Pre-Separation Filters, 30 µm, # 130-041-407) to remove cell clumps which may clog the column.

▲ 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. When working with **mouse** cells, resuspend cell pellet in 70 µL of buffer per 10^7 total cells.

   When working with **human** cells, resuspend cell pellet in 60 µL of buffer per 10^7 total cells.
4. (Optional) When working with **mouse** cells, add 10 µL of FcR Blocking Reagent, mouse per 10^7 total cells.

   (Optional) When working with **human** cells, add 20 µL of FcR Blocking Reagent, human per 10^7 total cells.
5. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
6. Add 20 µL of Anti-PSA-NCAM MicroBeads per 10^7 total cells. Mix well and incubate for 15 minutes 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 PSA-NCAM$^+$ cells. For details refer to table in section 1.4.

▲ To control the efficiency of the magnetic separation by flow cytometry, the appropriate volume of staining antibodies should first be added to the cell suspension after magnetic separation. For example, incubate 10^6 cells in 100 µL volume with 10 µL of PSA-NCAM-PE for 10 minutes in the dark in the refrigerator (2–8 °C).

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 appropriate amount of buffer:
   MS: 500 µL LS: 3 mL
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with the appropriate amount of buffer. Collect total effluent; this is the unlabeled cell fraction. Perform washing steps by adding buffer three times. Only add new buffer when the column reservoir is empty.
   MS: 3×500 µL LS: 3×3 mL
5. Remove column from the separator and place it on a suitable collection tube.
6. Pipette an 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 purity of PSA-NCAM$^+$ cells, the eluted fraction can be directly enriched over a second 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.

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 fraction collection tubes in rows B and C.
3. For a standard separation choose one of the following programs:

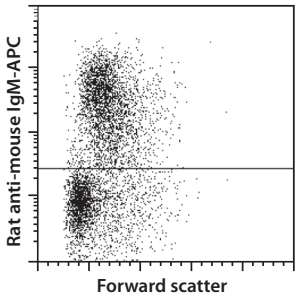
   Positive selection: Possel
   Collect positive fraction in row C of the tube rack.

   Depletion: Depl05
   Collect negative fraction in row B of the tube rack

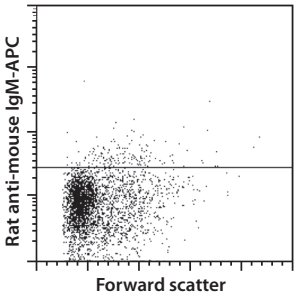
3. Example of a separation using Anti-PSA-NCAM MicroBeads

Separation of a single-cell suspension derived from day 1 postnatal mouse whole-brain tissue using the Neural Tissue Dissociation Kit (T), Anti-PSA-NCAM MicroBeads, a MiniMACS™ Separator, and two MS Columns. Cells were fluorescently stained with an APC-conjugated rat anti-mouse IgM antibody and analyzed by flow cytometry. Cell debris and dead cells are excluded from the analysis based on scatter signals and propidium iodide fluorescence.

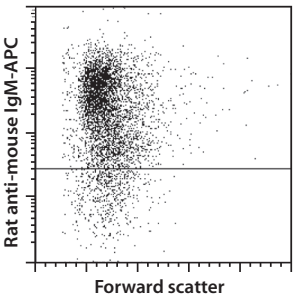
Original cell fraction



PSA-NCAM$^-$ cells



PSA-NCAM$^+$ cells



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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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