Miltenyi Biotec

# Anti-PSA-NCAM MicroBead Kit
## human and mouse

Order no. 130-097-859

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## 1. Description

| | |
|---|---|
| **Components** | **1 mL Anti-PSA-NCAM-APC, human and mouse:** monoclonal PSA-NCAM antibody conjugated to APC (clone: 2-2B; isotype: mouse IgM).<br>**2 mL Anti-APC MicroBeads:** MicroBeads conjugated to monoclonal anti-APC antibodies (isotype: mouse IgG1). |
| **Capacity** | For $10^9$ total cells, up to 100 separations. |
| **Product format** | All reagents 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 pluripotent stem cell-derived PSA-NCAM$^+$ cells are indirectly magnetically labeled with Anti-PSA-NCAM-APC and Anti-APC 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

The Anti-PSA-NCAM MicroBead Kit has been developed for the separation of human or mouse pluripotent stem cell-derived neuronal precursor cells based on the expression of the PSA-NCAM antigen. The Anti-PSA-NCAM antibody recognizes an epitope on polysialic acid (PSA) which, in vertebrates, is found 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 vitro* differentiation of human and mouse pluripotent stem cells under defined conditions revealed that neuronal progenitors, e.g. long-term self-renewing neuroepithelial-like stem cells, express the surface marker PSA-NCAM.[3] Magnetically enriched, *in vitro* generated PSA-NCAM positive neural progenitors can be further differentiated into defined neuronal subtypes.

### 1.3 Applications

- Positive selection of PSA-NCAM$^+$ cells derived from mouse or human pluripotent stem cells.

### 1.4 Reagent and instrument requirements

- DPBS buffer: Dulbecco's phosphate-buffered saline (DPBS) without $Ca^{2+}$ and $Mg^{2+}$.
- PB-Buffer: Dulbecco's phosphate-buffered saline (DPBS) without $Ca^{2+}$ and $Mg^{2+}$ and 0.5% bovine serum albumin (BSA). Keep buffer cold (2–8 °C). Degas buffer before use, as air bubbles could block the column.
- MACS Columns and MACS Separators: For optimal purity and recovery the use of an LS Column is strongly recommended. Positive selection 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 |
|---|---|---|---|
| **Positive selection** | | | |
| LS | $10^8$ | $2\times10^9$ | MidiMACS, QuadroMACS, SuperMACS II |
| autoMACS | $2\times10^8$ | $4\times10^9$ | autoMACS Pro, autoMACS |

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

- (Optional) CellMates™ Accumax™ Cell Dissociation Solution (# 130-095-546)
- (Optional) Trypsin solution: 0.05% trypsin, 2 mM EDTA.
- (Optional) Pre-Separation Filters, 70 µm (# 130-095-823) to remove cell clumps.
- (Optional) 5 mL serological pipette.
- (Optional) MACSmix™ Tube Rotator (# 130-090-753).


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# 2. Protocol

## 2.1 Sample preparation

Below mentioned differentiation protocols have been used to optimize enrichment of neuronal precursors derived from pluripotent stem cells using the Anti-PSA-NCAM MicroBead Kit.

**Preparation of mouse cells**
Differentiation of mouse pluripotent stem cells followed the protocol published by Lee *et al.*[4]
In brief, $2\times10^6$ pluripotent stem cells were differentiated as embryoid bodies (EBs) in DMEM and 5% fetal calf serum (FCS) and 5% serum replacement (SR). After 4 days, EBs were plated onto gelatine-coated dishes in DMEM and 5% FCS and 5% SR. After 24 hours, medium was changed to DMEM and Insulin-Transferrin-Selenium supplement and Fibronectin (5 µg/mL).

After additional 4–6 days of differentiation cells were harvested as followed:

1. Remove differentiation medium and wash culture plates twice with DPBS buffer.
2. Detach cells by addition of 2 mL of CellMates™ Accumax™ Cell Dissociation Solution per 10 cm culture dish for 15 minutes at 37 °C.
3. Stop enzymatic reaction by addition of 2 mL of DMEM and 5% FCS and 5% SR to the culture plate and dissociate cell clumps into single cells by pipetting up and down using a 1000 µL pipette.
4. Transfer cell suspension to an appropriate vial.
5. Pass cells through a 70 µm nylon mesh (Pre-Separation Filters, # 130-095-823) to remove cell clumps which may clog the column. Moisten filter with buffer or culture medium before use.

**Preparation of human cells**
Differentiation of human pluripotent stem cells followed the protocols published by Chambers *et al.*[5] and Zhou et *al.*[6].
In brief, $2\times10^6$ pluripotent stem cells were plated on Matrigel-coated 10 cm dishes in DMEM/F12 medium supplemented with 20% serum replacement, dorsomorphin (1 µM) and thiazovivin (2 µM).

After 10 days of differentiation cells were harvested as followed:

1. Remove differentiation medium and wash culture plates twice with DPBS buffer.
2. Detach cells by addition of 2 mL of trypsin solution (0.05% trypsin) per 10 cm culture dish for 5 minutes at 37 °C.
3. Stop enzymatic reaction by addition of soybean trypsin inhibitor.
4. Dissociate to single-cell suspension by pipetting up and down using a 5 mL serological pipette.
5. Pass cells through 70 µm nylon mesh (Pre-Separation Filters, # 130-095-823) to remove cell clumps which may clog the column. Moisten filter with buffer or culture medium before use.



## 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 $2\times10^7$ 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 70 µm nylon mesh (Pre-Separation Filters, 70 µm # 130-095-823) 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 5 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 100 µL of PB buffer per $10^7$ total cells.
4. Add 10 µL of Anti-PSA-NCAM-APC per $10^7$ total cells
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).
   ▲ **Note:** (Optional) The MACSmix Tube Rotator can be used for continuous mixing of larger volumes. Operate MACSmix Tube Rotator on permanent run at a speed of approximately 4 rpm.
6. Wash cells by adding 1–2 mL of PB buffer per $10^7$ cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. Resuspend cell pellet in 80 µL of PB buffer per $10^7$ total cells.
8. Add 20 µL of Anti-APC MicroBeads per $10^7$ total cells.
9. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
   ▲ **Note:** (Optional) The MACSmix Tube Rotator can be used for continuous mixing of larger volumes. Operate MACSmix Tube Rotator on permanent run at a speed of approximately 4 rpm.
10. Wash cells by adding 1–2 mL of PB buffer per $10^7$ cells and centrifuge at 300×g for 5 minutes. Aspirate supernatant completely.
11. Resuspend up to $10^7$ cells in 500 µL of PB buffer.
    ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
12. Proceed to magnetic separation (2.3).

### 2.3 Magnetic separation

▲ Choose one of the autoMACS Separators or an LS Column with an appropriate MACS Separator. For details refer to the table in section 1.4.

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

**Magnetic separation with 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 LS Column by rinsing with 3 mL of PB buffer.
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.
4. Wash column with 3×3 mL of PB buffer. Collect unlabeled cells that pass through and combine with the flow-through from step 3.

   ▲ **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 5 mL of PB buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.

**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 ≥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 of human cells: posselds**
   **Positive selection of mouse cells: possel**
   Collect positive fraction in row C of the tube rack.

**Magnetic separation with the autoMACS® Separator**

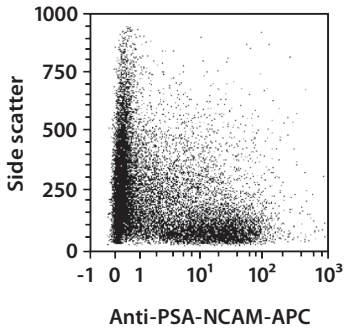
1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube at the uptake port and the fraction collection tubes at port neg1 and port pos1 (human cells) or pos2 (mouse cells).
3. For a standard separation choose one of the following programs:

   **Positive selection of human cells: posselds**
   **Positive selection of mouse cells: possel**
   Human cells: Collect positive fraction from outlet port pos1.
   Mouse cells: Collect positive fraction from outlet port pos2.

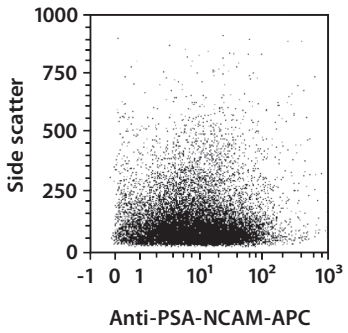
## 3. Example of a separation using the Anti-PSA-NCAM MicroBead Kit

PSA-NCAM$^+$ cells were isolated from human pluripotent stem cell-derived neuronal precursor cells using the Anti-PSA-NCAM MicroBead Kit, an LS Column, and a MidiMACS™ Separator. Cells were analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

Before separation




PSA-NCAM$^+$ cells



## 4. References


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3. Falk, A. *et al.* (2012) Capture of neuroepithelial-like stem cells from pluripotent stem cells provides a versatile system for *in vitro* production of human neurons. PLoS One 7: e29597.
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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.

### 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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