Miltenyi Biotec

Neural Crest Stem Cell MicroBeads
human

Order no. 130-097-127

Contents

1. Description

Components	2 mL Neural Crest Stem Cell MicroBeads, human: MicroBeads conjugated to monoclonal anti-human CD271 antibodies (isotype: mouse IgG1).
Capacity	For 10^9 total cells, up to 100 separations.
Product format	Neural Crest Stem Cell 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.

Cross-reactivity: The CD271 antibody also recognizes the CD271 antigen in monkey, goat, dog, pig, and sheep.[1]

1.1 Principle of the MACS® Separation

First, the CD271$^+$ neural crest stem cells are magnetically labeled with Neural Crest Stem Cell 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 CD271$^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of neural crest stem cells. After removing the column from the magnetic field, the magnetically retained CD271$^+$ cells can be eluted as the positively selected cell fraction. To increase the purity, the positively selected cell fraction containing the CD271$^+$ neural stem cells can be separated over a second column.

1.2 Background information

CD271, also known as LNGFR (low-affinity nerve growth factor receptor), NGFR (nerve growth factor receptor), or p75NTR (neurotrophin receptor), belongs to the tumor necrosis factor receptor superfamily. CD271 was initially described to be expressed on cells of the nervous system and was suggested to be involved in the development, survival and differentiation of neural cells.[2] CD271 can be found in the central and peripheral nervous system on autonomic and sensory neurons[3] as well as on glial cells, including oligodendrocytes[4], astrocytes[5], Schwann cells[6,7], and neural crest stem cells[8].

Neural crest stem cells can be induced from pluripotent stem cells by synergistic action of two inhibitors of SMAD signaling, Noggin and SB431542[9,10] or alternatively, by a single small molecule dorsomorphin blocking signalling of several (TGF-β) superfamily receptors[11]. CD271$^+$ neural crest stem cells can be selected ten days post-induction. They show expression of typical neural crest markers (HNK1, AP2) and they can be differentiated to homogenous peripheral neurons[12].

1.3 Applications

- Positive selection or depletion of neural crest stem cells differentiated from pluripotent stem cells. A method for *in vitro*–differentiation of neural crest stem cells from human iPS cells has been published and is available at www.macs-stemcells.com/downloads .

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 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: CD271$^+$ neural crest stem cells can be enriched by using MS Columns or depleted with the use of LD 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
Positive selection			
MS	10^7	2×10^7	MiniMACS, OctoMACS, SuperMACS II
Depletion			
LD	1×10^7	3×10^7	MidiMACS, QuadroMACS, SuperMACS II
Positive selection or depletion			
autoMACS	5×10^7	10^8	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.

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- (Optional) Fluorochrome-conjugated CD271 (LNGFR) antibodies for flow cytometric analysis, e.g., CD271 (LNGFR)-PE. 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.
- (Optional) Dead Cell Removal Kit (# 130-090-101) for the depletion of dead cells.
- (Optional) Pre-Separation Filters, 30 µm (# 130-041-407) to remove cell clumps.

2. Protocol

2.1 Sample preparation

Harvesting of neural crest stem cells differentiated from human embryonic stem cells (ES) or induced pluripotent cells (iPS)

A method for *in vitro*–differentiation of neural crest stem cells from human iPS cells has been published and is available at www.macs-stemcells.com/downloads.

1. Remove culture medium and wash culture plates twice with DPBS.
2. Trypsinize with 2 mL of trypsin solution per 10 cm culture dish for 5 minutes at 37 °C.
3. Stop enzymatic reaction by addition of 8 mL of culture medium containing fetal bovine serum (FBS) or trypsin inhibitor.
4. Dissociate to single-cell suspension by pipetting up and down using a 10 mL serological pipette.
5. (Optional) Pass cells through 30 µm nylon mesh (Pre-Separation Filters, 30 µm # 130-041-407) 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 2×10^7 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 (Pre-Separation Filters, 30 µm # 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. 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 Neural Crest Stem Cell 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 CD271 (LNGFR)-PE, and incubate for 5 minutes in the dark in the refrigerator (2–8 °C).
7. (Optional, for positive isolation) Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.

   ▲ **Note:** For depletion with LD Columns, autoMACS Pro Separator, or autoMACS Separator, the washing step should be omitted.
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 separation strategy and the number of total cells and the number of $CD271^+$ cells. 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 MS 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 500 µL of buffer.
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.
4. Wash column with 3×500 µL of 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 1 mL of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.
7. (Optional) To increase the purity of $CD271^+$ cells, the eluted fraction can be enriched over a second MS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

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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 flow-through; 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 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: Posseld2
   Collect positive fraction in row C of the tube rack.
   ▲ **Note:** Autolabeling is not possible.

   Depletion: Depletes
   Collect negative fraction in row B of the tube rack.
   ▲ **Note:** The use of autolabeling is recommended.

Magnetic separation with the autoMACS® Separator

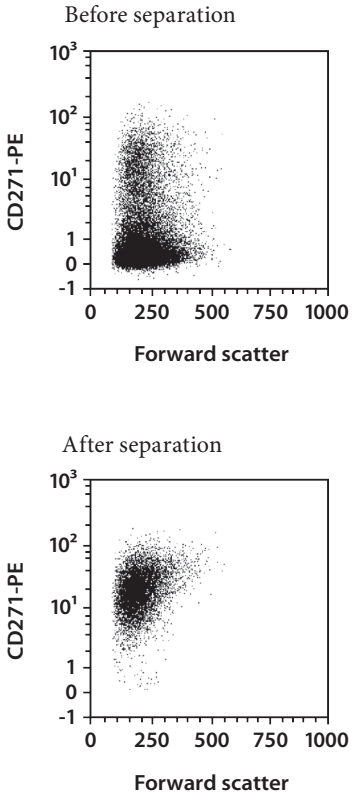
1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube at the uptake port and the fraction collection tubes at port neg1 and pos1.
3. For a standard separation choose one of the following programs:

   Positive selection: Posseld2
   Collect positive fraction from outlet port pos1.

   Depletion: Depletes
   Collect negative fraction from outlet port neg1.

3. Example of a separation using Neural Crest Stem Cell MicroBeads

Neural crest stem cells were *in vitro*–differentiated from human iPS cells using dorsomorphin for 10 days (10, 11) and subsequently isolated using Neural Crest Stem Cell MicroBeads, two MS Columns, and a MiniMACS™ Separator. Cells were fluorescently stained with CD271 (LNGFR)-PE and 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.



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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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