CD271 MicroBead Kit
human

Order no. 130-099-023

Contents

1. Description

Components	**2 mL CD271 MicroBeads, human:** MicroBeads conjugated to monoclonal CD271 antibodies (isotype: mouse IgG1). **2 mL FcR Blocking Reagent, human:** Human IgG.
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.

Cross-reactivity: CD271 MicroBeads have been reported to react with monkey, dog, pig, sheep, and goat cells.[11]

1.1 Principle of the MACS® Separation

First, the CD271$^+$ cells are magnetically labeled with CD271 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 CD271$^+$ 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$^+$ cells must be separated over a second column.

1.2 Background information

CD271, also known as LNGFR (low-affinity nerve growth factor receptor) or p75NTR, belongs to the low-affinity neurotrophin receptor and the tumor necrosis factor receptor superfamily.
CD271 is a well-known marker on mesenchymal stem cells, also known as mesenchymal stromal cells (MSCs), from bone marrow aspirate[1–4] or lipoaspirate[5]. After separation colony-forming unit fibroblast (CFU-F) activity was found only in the CD271$^+$ cell fraction, and not in the CD271$^-$ population[6–8]. Isolated CD271$^+$ cells have a higher proliferative capacity in comparison to MSCs isolated by plastic adherence[6,8,9]. Secretion of growth factors was significantly higher in the separated CD271$^+$ MSCs[7].
CD271 (LNGFR) was initially described to be expressed on cells of the central and peripheral nervous system nervous system and was suggested to be involved in the development, survival, and differentiation of neural cells[10]. CD271$^+$ neural crest progenitor cells can be efficiently isolated with Neural Crest Stem Cell MicroBeads.

1.3 Applications

- Positive selection or depletion of CD271$^+$ MSCs, e.g., from bone marrow or lipoaspirate.

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.

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- MACS Columns and MACS Separators: CD271$^+$ cells can be enriched by using MS or LS Columns or depleted with the use of LD Columns. Cells which strongly express the CD271 antigen can also be depleted using MS or LS 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	2×10^8	MiniMACS, OctoMACS, VarioMACS, SuperMACS II
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
Depletion			
LD	10^8	5×10^8	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
Positive selection or depletion			
autoMACS	2×10^8	4×10^9	autoMACS Pro, autoMACS

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

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD271 (LNGFR)-PE (# 130-091-885), CD271 (LNGFR)-APC (# 130-091-884), or CD45-FITC (# 130-080-202). 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.
- (Optional) StemMACS MSC Expansion Media (# 130-091-680).

2. Protocol

2.1 Sample preparation

When working with anticoagulated peripheral blood or buffy coat, peripheral blood mononuclear cells (PBMCs) should be isolated by density gradient centrifugation, for example, using Ficoll-Paque™.

▲ **Note:** To remove platelets after density gradient separation, resuspend cell pellet in buffer and centrifuge at 200×g for 10–15 minutes at 20 °C. Carefully aspirate supernatant. Repeat washing step.

When working with tissues or lysed blood, prepare a single-cell suspension using standard methods.

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

▲ 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 2×10^7 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.

▲ If CD271$^+$ MSCs are intended to be taken into culture, it is recommended to start with at least 2×10^7 bone marrow mononuclear cells (BM MNCs) in order to obtain sufficient cell numbers for subsequent cultivation of CD271$^+$ cells.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 60 µL of buffer per 10^7 total cells.
4. Add 20 µL of FcR Blocking Reagent and 20 µL of CD271 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 (# 130-091-885), 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).

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2.3 Magnetic separation

▲ Choose an appropriate MACS Column and MACS Separator according to 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 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 flow-through 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 CD271$^+$ 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:** For subsequent cell culture CD271$^+$ MSCs can be eluted in 1 mL StemMACS MSC Expansion Media (# 130-091-680). For seeding densities in CFU-F assays or MSC expansion culture refer to table 1.

Activity	CFU-F assay	MSC expansion	MSC expansion
Vessel	6 well	T25	T175
Surface area [cm^2]	9.6	25	175
StemMACS MSC Expansion Media [mL]	3	5	30
Total cell number [×10^5]	0.6–0.8	1.5–2.0	10.0–14.0

Table 1: Seeding density for CFU-F assay or MSC expansion.

2.4 Autolabeling and separation using 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 or the autoMACS Separator should have a temperature of ≥10 °C.

▲ For appropriate resuspension volumes and cell concentrations, please visit www.automacspro.com/autolabeling.

2.4.1 Autolabeling with the autoMACS® Pro Separator using the 2D code reader

1. Prepare and prime the instrument.
2. Go to the Reagent menu and highlight the position where the reagent vial will be placed in the MACS Reagent Rack. Four positions are available.
3. Select Read Reagent to activate the 2D code reader.
4. Present the vial in front of the 2D code reader.
5. Select reagent name appearing on screen.
6. After scanning the vial, the next available MACS Reagent Rack position will be automatically highlighted.
7. Insert the vial into the appropriate rack position.
8. Proceed to magnetic separation (2.4.2).

2.4.2 Magnetic separation

1. Highlight the desired position(s) in the sample separation template.
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. Choose reagent name from the labeling submenu.
4. (Optional) The recommended cell separation and wash program will be automatically displayed after choosing the program. It is possible to change the separation program or the wash program between samples or to assign the Sleep program after finishing the last sample. Highlight the desired cell separation and wash program in the Separation and Wash submenus, respectively.
5. Insert sample volume in the Volume submenu using the numeric keypad.
6. Select Run to start the cell separation.

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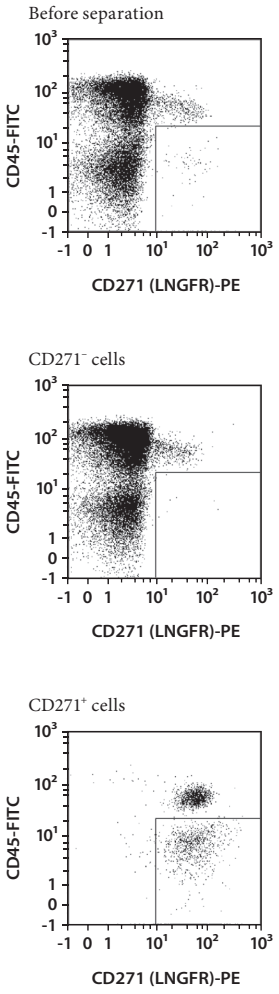
3. Example of a separation using the CD271 MicroBead Kit

$CD271^+$ cells were isolated from 1×10^7 bone marrow mononuclear cells (BM MNCs) by positive selection using two MS Columns. $CD271^+$ cells were detected based on CD271 (LNGFR)-PE (# 130-091-885) labeling and counterstaining with CD45-FITC (# 130-080-202). Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence. Among the isolated $CD271^+$ cells, the gated $CD45^-$ fraction exhibits clonogenic potential in CFU-F assay, whereas the $CD45^+$ fraction shows no proliferation in culture and will be lost during passaging[4].



4. References

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All protocols and data sheets are available at **www.miltenyibiotec.com**.

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.

Warranty

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