

Contents

1. Description
 - 1.1 Principle of the MACS® Separation
 - 1.2 Background information
 - 1.3 Applications
 - 1.4 Reagent and instrument requirements
2. Protocol
 - 2.1 Sample preparation
 - 2.2 Magnetic labeling
 - 2.3 Magnetic separation
3. Example of a separation using the CD127 MicroBead Kit
4. References

1. Description

Components	<p>1 mL CD127 Biotin, human: monoclonal anti-human CD127 antibody conjugated to Biotin (clone: MB15-18C9, isotype: mouse IgG2a).</p> <p>2 mL Anti-Biotin MicroBeads: MicroBeads conjugated to monoclonal anti-biotin antibodies.</p> <p>2 mL FcR Blocking Reagent, human</p>
Capacity	For 10 ⁹ total cells, up to 100 separations.
Product format	All components 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.

The CD127 antibody has been tested to cross-react with rhesus monkey (*Macaca mulatta*) and cynomolgus monkey (*Macaca fascicularis*).

1.1 Principle of the MACS® Separation

Peripheral blood mononuclear cells (PBMCs) are blocked with FcR Blocking Reagent and subsequently labeled with CD127-Biotin antibody and Anti-Biotin 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 CD127⁺ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of CD127⁺ cells. After removing the column from the magnetic field, the magnetically retained CD127⁺ cells can be eluted as the positively selected cell fraction.

1.2 Background information

CD127, the α -chain of the IL-7 receptor, is a type I membrane glycoprotein. Signalling of IL-7 through the IL-7R requires both IL-7Ra and the common cytokine gamma chain (γ c).¹ CD127 can

be identified on immature B cells through the early pre-B stage, on thymocytes, and on most mature T cells with transient down-regulation upon activation.^{2,3} CD127 is also used by thymic stromal-derived lymphopoietin (TSLP) as part of a complex.¹ On regulatory T cells CD127 is absent⁴ and its expression is inversely correlated with FoxP3 expression and suppressive function.^{5,6}

1.3 Applications

- Isolation or depletion of CD127⁺ T cells.
- Depletion of CD127⁺ T cells for the enrichment of regulatory T cells. Human regulatory T cells can be isolated, for example, by using the CD4⁺CD25⁺CD127^{dim/-} Regulatory T Cell Isolation Kit, human (# 130-094-775).

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 Ca²⁺ or Mg²⁺ are not recommended for use.

- MACS Columns and MACS Separators: CD127⁺ 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 CD127 antigen can also be depleted using MS, LS, or XS 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
LS	10 ⁸	2×10 ⁹	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
XS	10 ⁹	2×10 ¹⁰	SuperMACS
Depletion			
LD	10 ⁸	5×10 ⁸	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
CS	2×10 ⁸		VarioMACS, SuperMACS
D	10 ⁹		SuperMACS
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™ Separators. For details see the respective MACS Separator data sheet.

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD4 (VIT4)-FITC (# 130-092-358), Anti-Biotin-PE (# 130-090-756), CD127-PE (# 130-094-889), and CD25-APC (# 130-092-858). For more information about fluorochrome-conjugated antibodies see www.miltenyibiotec.com.
- (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 (# 130-041-407) to remove cell clumps.

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 see 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 10⁷ total cells. When working with fewer than 10⁷ 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 2×10⁷ 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, # 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. Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 30 μL of buffer per 10⁷ total cells.
4. Add 10 μL of FcR Blocking Reagent and 10 μL of CD127-Biotin per 10⁷ total cells.

4. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
6. Add 30 μL of buffer per 10⁷ total cells.
7. Add 20 μL of Anti-Biotin MicroBeads per 10⁷ total cells.
8. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
9. (Optional) Add staining antibodies, e.g., 5 μL of CD4 (VIT4)-FITC (# 130-092-358) and 5 μL of Anti-Biotin-PE (# 130-090-756), and incubate for 5 minutes in the dark in the refrigerator (2–8 °C).
10. Wash cells by adding 1–2 mL of buffer per 10⁷ cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
11. Resuspend up to 10⁸ 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 1.25×10⁸ cells in 500 μL of buffer.
12. 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 CD127⁺ cells. For details see 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 see 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
----------	----------

Magnetic separation with XS Columns

For instructions on the column assembly and the separation refer to the XS Column data sheet.

Depletion with LD Columns

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

Depletion with CS Columns

1. Assemble CS Column and place it in the magnetic field of a suitable MACS Separator. For details see CS Column data sheet.
2. Prepare column by filling and rinsing with 60 mL of buffer. Attach a 22G flow resistor to the 3-way stopcock of the assembled column. For details see CS Column data sheet.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with 30 mL buffer from the top. Collect total effluent; this is the unlabeled cell fraction.

Depletion with D Columns

For instructions on column assembly and separation refer to the D Column data sheet.

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

Depletion: "Deplete"
Collect negative fraction in row B of the tube rack.

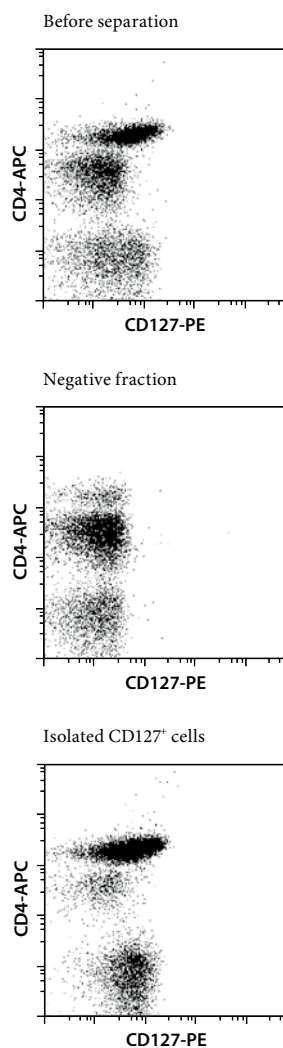
Magnetic separation with the autoMACS® Separator

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.
3. For a standard separation choose one of the following programs:
Positive selection: "Possel"
Collect positive fraction from outlet port pos1.

Depletion: "Deplete"
Collect negative fraction from outlet port neg1.

3. Example of a separation using the CD127 MicroBead Kit

CD127⁺ cells were isolated from human PBMCs using the CD127 MicroBead Kit, an MS Column, and a MiniMACS™ Separator. Cells were fluorescently stained with CD4 (VIT4)-FITC (# 130-092-358) and CD127-PE (# 130-094-889) and analyzed by using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



4. References

1. Fry, T.J. and Mackall, C.L. (2002) Interleukin-7: from bench to clinic. *Blood* 99: 3892–3904.
2. Sudo, T. *et al.* (1993) Expression and function of the interleukin 7 receptor in murine lymphocytes. *J. Immunol.* 90: 9125–9129.
3. Armitage, R.J. *et al.* (1991) Expression of receptors for interleukin 4 and interleukin 7 on human T cells. *Adv. Exp. Med. Biol.* 292: 121–130.
4. Cupedo, T. *et al.* (2005) Development and activation of regulatory T cells in the human fetus. *Eur. J. Immunol.* 35: 383–390.
5. Seddiki, N. *et al.* (2006) Expression of interleukin (IL)-2 and IL-7 receptors discriminates between human regulatory and activated T cells. *J. Exp. Med.* 203: 1693–1700.
6. Liu, W. *et al.* (2006) CD127 expression inversely correlates with FoxP3 and suppressive function of human CD4⁺ T reg cells. *J. Exp. Med.* 203: 1701–1711.

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

The products sold hereunder are warranted only to be free from defects in workmanship and material at the time of delivery to the customer. Miltenyi Biotec GmbH makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the technical specifications of the products. Miltenyi Biotec GmbH's liability is limited to either replacement of the products or refund of the purchase price. Miltenyi Biotec GmbH is not liable for any property damage, personal injury or economic loss caused by the product.

autoMACS, MACS, and MACSQuant are registered trademarks and MidiMACS, MiniMACS, OctoMACS, QuadroMACS, SuperMACS, and VarioMACS are trademarks of Miltenyi Biotec GmbH.

Ficoll-Paque is a trademark of GE Healthcare companies.

Copyright © 2009 Miltenyi Biotec GmbH. All rights reserved.