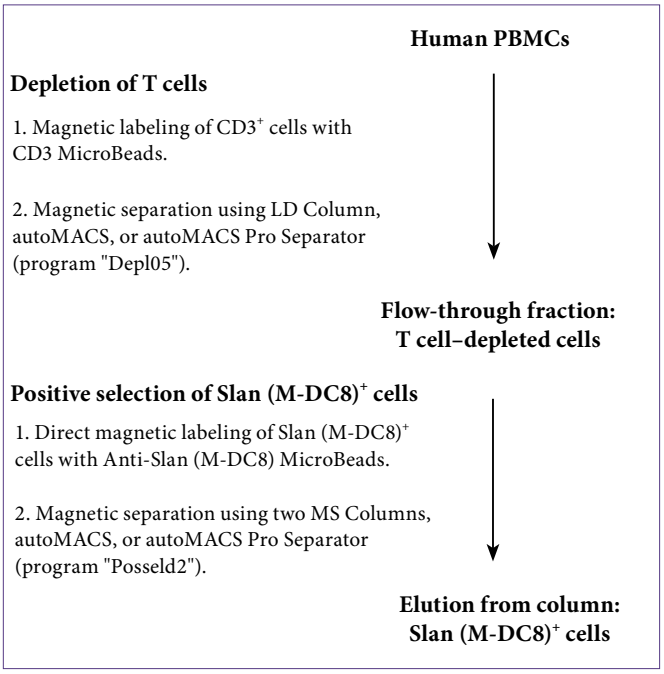
Slan (M-DC8)$^+$ Monocyte Isolation Kit
human

Order no. 130-093-026

Contents



1. Description

Components	**2 mL CD3 MicroBeads, human:** MicroBeads conjugated to monoclonal anti-human CD3 antibodies. **2 mL FcR Blocking Reagent, human.** **2 mL Anti-Slan (M-DC8) MicroBeads, human:** MicroBeads conjugated to monoclonal anti-human Slan (M-DC8) antibodies.
Capacity	For 2×10^9 total cells, up to 20 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.

1.1 Principle of the MACS® Separation

The Slan (M-DC8)$^+$ Monocyte Isolation Kit is a two-step magnetic labeling system for the isolation of Slan (M-DC8)$^+$ cells from human peripheral blood mononuclear cells (PBMCs). In the first step, CD3$^+$ cells are magnetically labeled by using CD3 MicroBeads. The labeled cells are subsequently depleted by separation over a MACS® Column.
In the second step, Slan (M-DC8)$^+$ cells are labeled with Anti-Slan (M-DC8) MicroBeads and isolated by positive selection from the T cell–depleted fraction. The magnetically labeled Slan (M-DC8)$^+$ cells are retained within the column and eluted after removal of the column from the magnetic field.

1.2 Background information

The Slan (M-DC8)$^+$ Monocyte Isolation Kit, human, was developed for the isolation of Slan (M-DC8)$^+$ cells from PBMCs.

Slan (6-Sulfo LacNAc) is a carbohydrate modification of P-selectin glycoprotein ligand-1 (PSGL-1) characteristically expressed on a new subset of PBMCs with features closely related to CD16$^+$CD14^{low} monocytes.[1,2]

Slan (M-DC8)$^+$ cells constitute 0.5–2% of all PBMCs with similar frequencies among mononuclear cells from cord blood.[3] Freshly isolated, Slan (M-DC8)$^+$ cells have an eminent capacity to produce TNF-α following stimulation with LPS.[4] Moreover, they are involved in antibody-dependent cell-mediated cytotoxicity.[5] Furthermore, Slan (M-DC8)$^+$ cells promote proliferation, IFN-γ–production, and tumor-directed cytotoxicity of NK cells.[6] Upon stimulation with IFN-γ, Slan (M-DC8)$^+$ cells possess the ability to directly lyse tumor cell lines *in vitro*.[6] Additionally, Slan (M-DC8)$^+$ cells are potent producers of early IL-12 upon stimulation.[7] Because of their highly proinflammatory phenotype, Slan (M-DC8)$^+$ cells might be important in several Th-1–dominated diseases like psoriasis and rheumatoid arthritis.

1.3 Application

- Isolation of Slan (M-DC8)$^+$ monocytes from PBMCs.

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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. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- MACS Columns and MACS Separators: Depletion of $CD3^+$ cells is performed on an LD Column. The subsequent positive selection of Slan $(M\text{-}DC8)^+$ cells is performed on MS Columns. Depletion and positive selection can also be performed by using the autoMACS or the autoMACS Pro Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Depletion			
LD	10^8	5×10^8	MidiMACS, OctoMACS, VarioMACS, SuperMACS
Positive selection			
MS	10^7	2×10^8	MiniMACS, QuadroMACS, VarioMACS, SuperMACS
Depletion and positive selection			
autoMACS	2×10^8	4×10^9	autoMACS, autoMACS Pro

▲ **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 Anti-Slan (M-DC8) antibody for flow cytometric analysis, e.g., Anti-Slan (M-DC8)-FITC (# 130-093-027), Anti-Slan (M-DC8)-PE (# 130-093-029), or Anti-Slan (M-DC8)-APC (# 130-093-031). For more information about other fluorochrome-conjugates see www.miltenyibiotec.com.
- (Optional) Propidium iodide (PI) 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

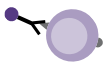
2.1 Sample preparation

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™. For details see the General Protocols section of the respective separator user manual. The General Protocols are also available at www.miltenyibiotec.com/protocols.

▲ **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 General Protocols section of the respective separator user manual. The General Protocols are also available 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 of $CD3^+$ cells

▲ 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 10^8 total cells. When working with fewer than 10^8 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 2×10^8 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, # 130-041-407) to remove cell clumps which may clog the column. Wet filter with buffer before use.

▲ 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 300 µL of buffer per 10^8 total cells.
4. Add 100 µL of FcR Blocking Reagent per 10^8 total cells.
5. Add 100 µL of CD3 MicroBeads per 10^8 total cells.
6. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
7. Wash cells by adding 5–10 mL of buffer per 10^8 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: Depletion of $CD3^+$ T cells

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 containing Slan (M-DC8)$^+$ cells. Perform washing steps by adding buffer two times. Only add new buffer when the column reservoir is empty.
5. Proceed to 2.4 for the isolation of Slan (M-DC8)$^+$ cells.

Depletion with the autoMACS™ Separator or the autoMACS™ Pro Separator

▲ Refer to the respective user manual for instructions on how to use the autoMACS™ Separator or the autoMACS Pro Separator.

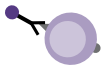
▲ Buffers used for operating the autoMACS Separator or the autoMACS Pro Separator should have a temperature of ≥ 10 °C.

Depletion 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 below the uptake port and the fraction collection tubes at port neg1 and port pos1.
3. Choose program "Depl05"
4. Collect negative fraction from outlet port neg1.
5. Proceed to 2.4 for the isolation of Slan (M-DC8)$^+$ cells.

Depletion 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 fraction collection tubes in rows B and C.
3. Choose program "Depl05"
4. Collect negative fraction in row B of the tube rack.
5. Proceed to 2.4 for the isolation of Slan (M-DC8)$^+$ cells.



2.4 Magnetic labeling of Slan (M-DC8)$^+$ cells

▲ Volumes for magnetic labeling given below are for an initial starting cell number of up to 10^8 cells. For larger initial cell numbers, scale up volumes accordingly.

1. Centrifuge cells at 300×g for 10 minutes. Aspirate supernatant completely.
2. Resuspend cell pellet in 400 µL of buffer per 10^8 initial cells.
3. Add 100 µL of Slan (M-DC8) MicroBeads per 10^8 initial cells.
4. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
5. Wash cells by adding 5–10 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
6. Resuspend up to 10^8 cells in 500 µL of buffer.
7. Proceed to magnetic separation (2.5).

2.5 Magnetic separation: Positive selection of Slan (M-DC8)$^+$ cells

Positive selection with MS Columns

▲ To achieve highest purities, perform two consecutive column runs.

1. Place MS Column in the magnetic field of a suitable MACS Separator. For details see MS Column data sheet.
2. Prepare column by rinsing with 500 µL of buffer.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells which pass through and wash column with 3×500 µL of buffer. Perform washing steps by adding buffer three times once the column reservoir is empty.
5. Remove column from the separator and place it on a suitable collection tube.

   ▲ **Note:** To perform a second column run, you may elute the cells directly from the first onto the second, equilibrated column instead of a collection tube.
6. Pipette 1 mL of buffer onto the column. Immediately flush out the fraction with magnetically labeled cells (Slan (M-DC8)$^+$ cells) by firmly applying the plunger supplied with the column.
7. To increase purity of Slan (M-DC8)$^+$ 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.

Positive selection with the autoMACS Separator

▲ Refer to the respective user manual for instructions on how to use the autoMACS™ Separator or the autoMACS Pro Separator.

▲ Buffers used for operating the autoMACS Separator or the autoMACS Pro Separator should have a temperature of ≥ 10 °C.

Positive selection 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 below the uptake port and the fraction collection tubes at port neg1 and port pos2.
3. Choose program "Posseld2"
4. Collect positive fraction from outlet port pos2.

Positive selection 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 fraction collection tubes in rows B and C.
3. Choose program "Posseld2"
4. Collect positive fraction in row C of the tube rack.

2.6 (Optional) Evaluation of Slan (M-DC8)$^+$ cell purity

The purity of the enriched Slan (M-DC8)$^+$ cells or any intermediate fraction can be evaluated by flow cytometry or fluorescence microscopy. Stain aliquots of the cell fractions with fluorochrome-

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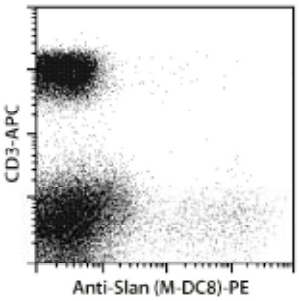
conjugated antibodies against Slan (M-DC8)$^+$, e.g., Anti-Slan (M-DC8)-FITC (# 130-093-027).

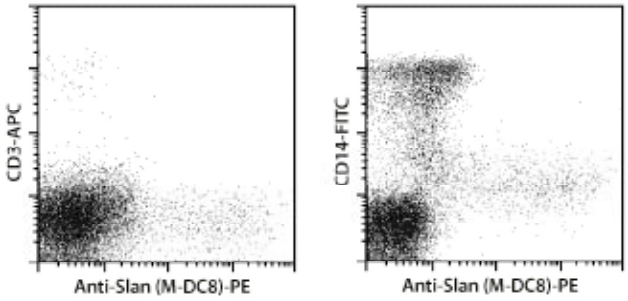
3. Example of a separation using the Slan (M-DC8)$^+$ Monocyte Isolation Kit

Slan (M-DC8)$^+$ cells were isolated from human PBMCs using the Slan (M-DC8)$^+$ Monocyte Isolation Kit, LD and MS Columns, and appropriate separators (see table in section 1.4). Cells are fluorescently stained with Anti-Slan (M-DC8)-PE (# 130-093-029), CD3-APC (# 130-091-373), or CD14-FITC (# 130-080-701). Cell debris and dead cells are excluded from the analysis based on scatter signals and PI fluorescence.

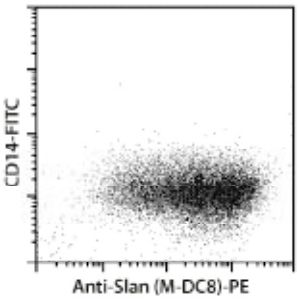
PBMCs before separation



T cell–depleted flow-through fraction



Isolated Slan (M-DC8)$^+$ monocytes



4. References

1. Schäkel, K. *et al.* (1998) A novel dendritic cell population in human blood: one-step immunomagnetic isolation by a specific mAb (M-DC8) and *in vitro* priming of cytotoxic T lymphocytes. Eur. J. Immunol. 28: 4084–4093.
2. Schäkel, K. *et al.* (2002) 6-Sulfo LacNAc, a novel carbohydrate modification of PSGL-1, defines an inflammatory type of human dendritic cells. Immunity 17: 289–301.
3. De Baey, A. *et al.* (2001) Phenotype and function of human dendritic cells derived from M-DC8$^+$ monocytes. Eur. J. Immunol. 31: 1646–1655.
4. De Baey, A. *et al.* (2003) A subset of human dendritic cells in the T cell area of mucosa-associated lymphoid tissue with a high potential to produce TNF-α. Eur. J. Immunol. 170: 5089–5094.
5. Schmitz, M. *et al.* (2002) Native human blood dendritic cells as potent effectors in antibody-dependent cellular cytotoxicity. Blood 100: 1502–1504.
6. Schmitz, M. *et al.* (2005) Tumoricidal potential of native blood dendritic cells: direct tumor cell killing and activation of NK cell-mediated cytotoxicity. J. Immunol. 174: 4127–4134.
7. Schäkel, K. *et al.* (2006) Human 6-sulfo LacNAc-expressing dendritic cells are principal producers of early interleukin-12 and are controlled by erythrocytes. Immunity 24: 767–777.

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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