



Anti-Slan (M-DC8) antibodies human

Anti-Slan (M-DC8)-FITC	130-093-027
Anti-Slan (M-DC8)-PE	130-093-029
Anti-Slan (M-DC8)-APC	130-093-031
Anti-Slan (M-DC8)-Biotin	130-093-033

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1. Description

Components	1 mL Anti-Slan (M-DC8) antibodies, human: monoclonal Anti-Slan (M-DC8) antibodies conjugated to fluorescein isothiocyanate (FITC), R-phycoerythrin (PE), allophycocyanin (APC), or biotin.
Clone	DD-1 (isotype: mouse IgM).
Capacity	100 tests or up to 10 ⁹ total cells.
Product format	Antibodies 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 Background information

Slan (6-Sulfo LacNAc) is a carbohydrate modification of P-selectin glycoprotein ligand-1 (PSGL-1) characteristically expressed on a new subset of peripheral blood mononuclear cells (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 blood³.

1.2 Applications

- Identification and enumeration of Slan (M-DC8)⁺ cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy. Human Slan (M-DC8)⁺ monocytes can be isolated by using the Slan (M-DC8)⁺ Monocyte Isolation Kit, human (# 130-093-026).

1.3 Recommended antibody dilution

For antibody labeling of human cells.

Anti-Slan (M-DC8) conjugate	FITC	PE	APC	Biotin
Flow cytometry^a				
- In general	1:11	1:11	1:11	1:11
- Formaldehyde-fixed cells ^b	1:11	1:11	1:11	1:11
- Anti-Slan (M-DC8) MicroBead-labeled cells	1:11	1:11	1:11	1:11

a) Given antibody dilutions are for a cell concentration of up to 10⁷ cells/100 µL of buffer.
b) For optimal results, cells must be stained prior to fixation.

1.4 Reagent 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).
▲ **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 Ca²⁺ or Mg²⁺ are not recommended for use.
- (Optional) FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor-mediated antibody labeling.
- (Optional) Anti-Biotin-FITC (# 130-090-857), Anti-Biotin-PE (# 130-090-756), or Anti-Biotin-APC (# 130-090-856) as secondary antibody reagent in combination with Anti-Slan (M-DC8)-Biotin.
- (Optional) Propidium iodide (PI) or 7-AAD for flow cytometric exclusion of dead cells without fixation.
- (Optional) Fixation and Dead Cell Discrimination Kit (# 130-091-163) for cell fixation and flow cytometric exclusion of dead cells.

2. General protocol for immunofluorescent staining

▲ Volumes given below are for up to 10⁷ nucleated 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 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10⁷ nucleated cells per 100 µL of buffer.

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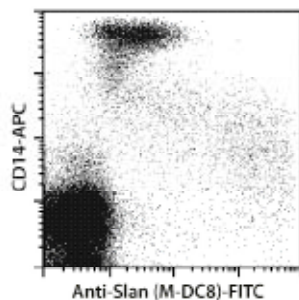
4. Add 10 μ L of the Anti-Slan (M-DC8) antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).

▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.
6. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at $300\times g$ for 10 minutes. Aspirate supernatant completely.
7. (Optional) If Anti-Slan (M-DC8)-Biotin was used, resuspend the cell pellet in 100 μ L of buffer, add 10 μ L of anti-biotin antibody (Anti-Biotin-FITC, Anti-Biotin-PE, or Anti-Biotin-APC), and continue as described in steps 5 and 6.
8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

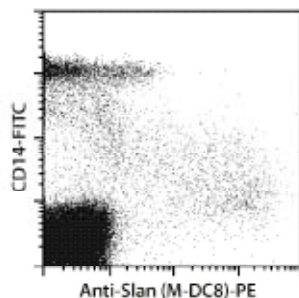
3. Examples of immunofluorescent staining with Anti-Slan (M-DC8) antibodies

Human PBMCs were stained with Anti-Slan (M-DC8) antibodies conjugated to FITC (a), PE (b), or APC (c) as well as with CD14 antibodies conjugated to FITC or APC and analyzed by flow cytometry. Cells stained with Anti-Slan (M-DC8)-Biotin (d) were stained with Anti-Biotin-APC (# 130-090-857) and CD14-FITC. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

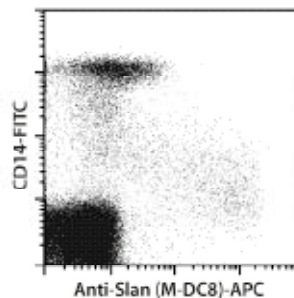
(a) Human PBMCs stained with Anti-Slan (M-DC8)-FITC and CD14-APC.



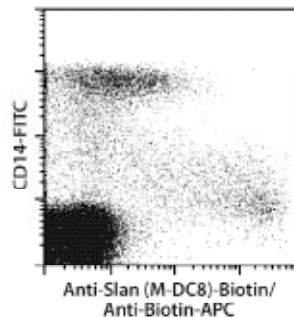
(b) Human PBMCs stained with Anti-Slan (M-DC8)-PE and CD14-FITC.



(c) Human PBMCs stained with Anti-Slan (M-DC8)-APC and CD14-FITC.



(d) Human PBMCs stained with Anti-Slan (M-DC8)-Biotin, Anti-Biotin-APC, and CD14-FITC.



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.

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.

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