Anti-Slan (M-DC8) antibodies, human

For research use only

One test corresponds to labeling of up to 10^7 cells in a total volume of 100 µL.

Product	Content	Order no.
Anti-Slan (M-DC8)-FITC	for 30 tests	130-099-128
Anti-Slan (M-DC8)-FITC	for 100 tests	130-093-027
Anti-Slan (M-DC8)-PE	for 100 tests	130-093-029
Anti-Slan (M-DC8)-APC	for 30 tests	130-099-189
Anti-Slan (M-DC8)-APC	for 100 tests	130-093-031
Anti-Slan (M-DC8)-VioBlue	for 30 tests	130-101-178
Anti-Slan (M-DC8)-VioBlue	for 100 tests	130-101-176
Anti-Slan (M-DC8)-Biotin	for 30 tests	130-100-680
Anti-Slan (M-DC8)-Biotin	for 100 tests	130-093-033

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.

Technical data and background information

Antigen	Slan (M-DC8)
Clone	DD-1
Isotype	mouse IgMκ
Isotype control	Mouse IgM – isotype control antibodies
Alternative names of antigen	SELPLG, CD162, CLA, PSGL-1, PSGL1
Product format	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.
Fixation	Cells should be stained prior to fixation, if formaldehyde is used as a fixative.
Storage	Store protected from light at 2–8 °C. Do not freeze.

The Anti-Slan (M-DC8) antibody (clone DD-1) recognizes Slan (6-Sulfo LacNAc), which is a carbohydrate modification of P-selectin glycoprotein ligand-1 (PSGL-1). This antigen is characteristically expressed on a new subset of peripheral blood mononuclear cells (PBMCs) with features closely related to $CD16^+CD14^{low}$ monocytes.

Slan $(M\text{-}DC8)^+$ cells constitute 0.5–2% of all PBMCs with similar frequencies among mononuclear cells from cord blood.

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). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.
- (Optional) FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor–mediated antibody labeling.
- (Optional) Fluorochrome-conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with biotinylated antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) 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.

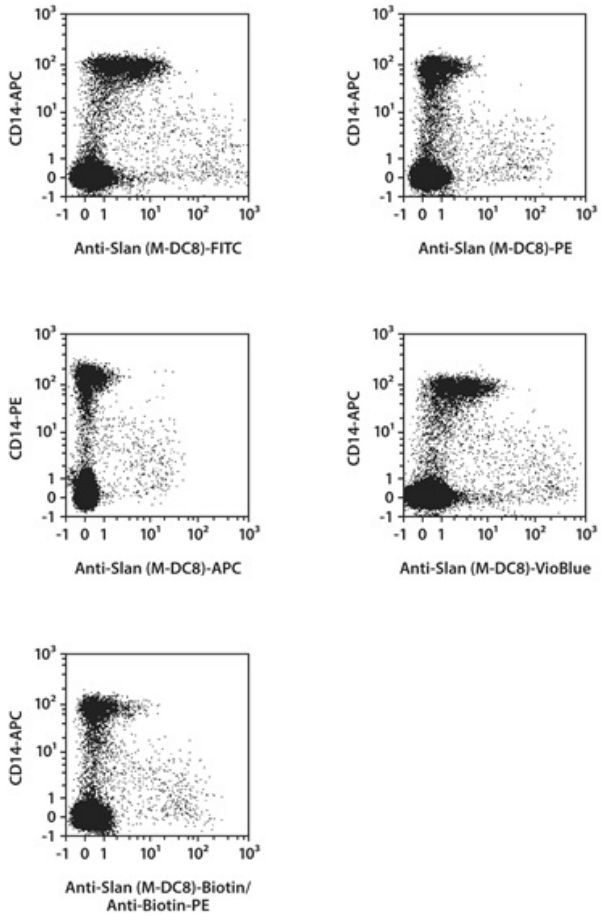
Protocol for cell surface staining

- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:11 for up to 10^7 cells/100 µL of buffer.
- Volumes given below are for up to 10^7 nucleated 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 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 10^7 nucleated cells per 100 µL of buffer.
4. Add 10 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   Note: Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.
6. Wash cells by adding 1−2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. (Optional) If biotinylated antibody was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL of fluorochrome-conjugated anti-biotin antibody, 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.

Examples of immunofluorescent staining

Human peripheral blood mononuclear cells (PBMCs) were stained with Anti-Slan(M-DC8) antibodies as well as with CD14 antibodies and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



References

1. **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.
2. **Akram, A. *et al.*** (2001) Phenotype and function of human dendritic cells derived from M-DC8^{+} monocytes. Eur. J. Immunol. 31: 1646–1655.
3. **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.

Warranty

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