



Antibodies

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1. Description

Clone	NLDC-145 (isotype: rat IgG2a).
Product format	1 mL CD205 (DEC205) antibodies, mouse: monoclonal CD205 (DEC205) antibodies conjugated to R-phycoerythrin (PE), allophycocyanin (APC), or biotin (Biotin). Antibodies are supplied in a solution containing stabilizer and 0.05% sodium azide.
Product size	100 tests (for up to 10 ⁹ total cells).
Storage	Store protected from light at 4–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Background and product applications

The CD205 antibody recognizes a 205 kDa integral membrane glycoprotein also known as DEC205 (dendritic and epithelial cells, 205 kDa).^{1,2} This membrane protein acts as an endocytic receptor and thereby mediates efficient processing and presentation of antigens *in vivo*, leading to induction of T cell immunity or tolerance.³ CD205 is expressed at high levels on mouse dendritic cells (DCs) in the skin (Langerhans cells), on DCs residing in the T cell areas of peripheral lymphoid organs, and on DCs generated *in vitro* from bone marrow progenitors.⁴ To a much lower extent (10–50× lower), CD205 is also expressed on mature B cells, granulocytes and T cells.^{1,2}

Product applications

- Identification and enumeration of CD205⁺ cells by flow cytometry or fluorescence microscopy.
- Staining of dendritic cells in combination with other markers like CD11c, CD8, or MHC class II for subset specific analysis.

CD205 (DEC205) antibodies mouse

CD205 (DEC205)-PE	130-092-286
CD205 (DEC205)-APC	130-092-285
CD205 (DEC205)-Biotin	130-092-468

1.2 Examples of staining concentrations for mouse cells.

CD205 (DEC205) conjugate	PE	APC	Biotin
	Recommended antibody dilution		
Flow cytometry^a			
- in general	1:11	1:11	1:11
- formaldehyde-fixed cells	1:11	1:11	n.r.
a) Given antibody dilutions are for a cell concentration of up to 1×10 ⁸ cells/mL buffer n.r. not recommended			

1.3 Reagent requirements

- Buffer: Prepare a solution containing PBS (phosphate-buffered saline) pH 7.2, 0.5% BSA (bovine serum albumin) and 2 mM EDTA, e.g. by diluting MACS® BSA Stock Solution (# 130-091-376) 1:20 with autoMACS™ Rinsing Solution (# 130-091-222). Keep buffer cold (4–8 °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 mouse serum albumin, mouse serum, or fetal calf serum. Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- (Optional) Fc receptor blocking reagent (CD16/32 monoclonal antibody) to avoid Fc receptor-mediated fluorescent staining
- (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 CD205 (DEC205)-Biotin.
- (Optional) PI (propidium iodide) or 7-AAD for flow cytometric exclusion of dead cells without cell fixation. For cell fixation and flow cytometric exclusion of dead cells, the Fixation and Dead Cell Discrimination Kit (# 130-091-163) is recommended.

2. General protocol for immunofluorescent staining

▲ Volumes for fluorescent labeling 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. Resuspend up to 10⁷ nucleated cells per 100 µL of buffer.
2. Add 10 µL of CD205 (DEC205) antibodies.
▲ Note: See table for exceptions.
3. Mix well and incubate for 10 minutes in the dark at 4–8 °C.
▲ Note: Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.
4. Wash cells by adding 1–2 mL of buffer per 10⁷ cells and centrifuge at 300×g for 10 minutes. Pipette off supernatant completely.

140-001-533-01

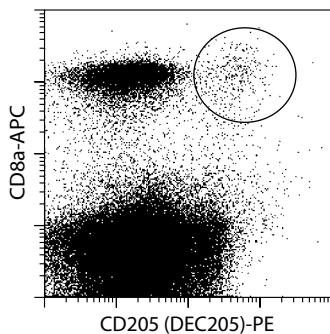


5. (Optional) If CD205 (DEC205)-Biotin was used, resuspend the cell pellet in 100 μ L buffer, add 10 μ L Anti-Biotin antibody (Anti-Biotin-FITC #130-090-857, Anti-Biotin-PE #130-090-756, or Anti-Biotin-APC #130-090-856), and continue as described in step 3 and 4.
6. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

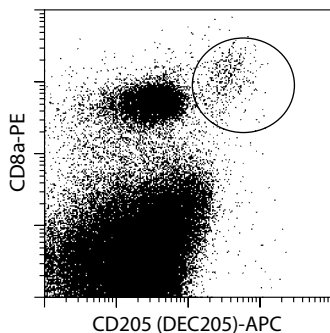
3. Examples of immunofluorescent staining with CD205 (DEC205) antibodies

Mouse spleen cells were stained with CD11c- and CD8-conjugated antibodies as well as with CD205 (DEC205) antibodies conjugated to PE (a), APC (b), or biotin (c), respectively. Samples were then analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

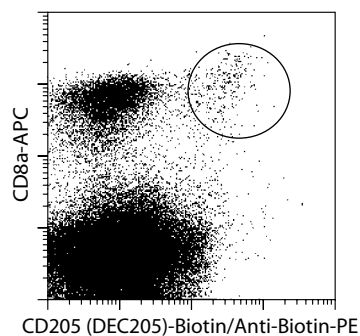
(a) Mouse spleen cells stained with CD205 (DEC205)-PE.



(b) Mouse spleen cells stained with CD205 (DEC205)-APC.



(c) Mouse spleen cells stained with CD205 (DEC205)-Biotin.



4. References

1. Inaba, K. *et al.* (1995) Tissue distribution of the DEC-205 protein that is detected by the monoclonal antibody NLDC-145. I. Expression on dendritic cells and other subsets of mouse leukocytes. *Cell. Immun.* 163: 148–156.
2. Witmer-Pack, M. D. *et al.* (1995) Tissue distribution of the DEC-205 protein that is detected by the monoclonal antibody NLDC-145. II. Expression *in situ* in lymphoid and nonlymphoid tissues. *Cell. Immun.* 163: 157–162.
3. Bonifaz, L. *et al.* (2002) Efficient targeting of protein antigen to the dendritic cell Receptor DEC-205 in the steady state leads to antigen presentation on major histocompatibility complex class I products and peripheral CD8⁺ T cell tolerance. *J. Exp. Med.* 196: 1627–1638.
4. Kraal, G. *et al.* (1986) Langerhans cells, veiled cells, and interdigitating cells in the mouse recognized by a monoclonal antibody. *J. Exp. Med.* 163: 981–997.

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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