CD226 (DNAM-1) antibodies
human

CD226 (DNAM-1)-PE	130-092-476
CD226 (DNAM-1)-APC	130-092-477
CD226 (DNAM-1)-Biotin	130-092-478
CD226 (DNAM-1) pure	130-092-479

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1. Description

Components 1 mL CD226 (DNAM-1), human: monoclonal CD226 (DNAM-1) antibodies conjugated to R-phycoerythrin (PE), allophycocyanin (APC), or biotin (Biotin). The unconjugated (pure) antibody is supplied at a concentration of 100 µg/mL.

Clone DX11 (isotype: mouse IgG1)

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

CD226 (DNAM-1) is a 65 kDa transmembrane glycoprotein[1] consisting of two Ig-like domains in the extracellular portion and a cytoplasmic tail containing three tyrosine residues[2]. CD226 (DNAM-1) is known to be expressed on NK cells, T cells, monocytes and a small portion of B cells[3] in addition to activated platelets and megakaryocytes, where it has been shown to be involved in their adhesion to endothelial cells[4].

The surface association of CD226 (DNAM-1) with LFA-1 on NK cells and T cells permits the transduction of cytolytic signals upon binding of CD226 (DNAM-1) to its ligands, CD155 (polio virus receptor, PVR), CD112 (Nectin-2), and CD96 (Tactile).[2] These ligands have also been shown to be recognized in the NK cell-mediated lysis of neoplastic cells.[5,6] As with other NK cell cytolytic triggering receptors, cytotoxic activity is regulated by HLA-specific inhibitory receptors.[3]

1.2 Applications

- Identification and enumeration of CD226 (DNAM-1)$^+$ cells by flow cytometry or fluorescence microscopy.
- Phenotypic analysis of NK cells by flow cytometry or fluorescence microscopy after MACS® Separation. Human NK cells can be isolated by using for example, the NK Cell Isolation Kit, human (# 130-092-657).

1.3 Recommended antibody dilution

For antibody labeling of human cells.

CD226 (DNAM-1) conjugate	PE	APC	Biotin
Flow cytometry[a]			
- in general	1:11	1:11	1:11
- formaldehyde-fixed cells[b]	1:11	1:11	1:11

a) Given antibody dilutions are for a cell concentration of up to 10^7 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, e.g., 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 calf serum. 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 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 CD226 (DNAM-1)-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 for fluorescent labeling 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 (for example, 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 CD226 (DNAM-1) antibodies.

140-001-602.02

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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 lead to non-specific cell labeling.

6. Wash cells by adding 1−2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.

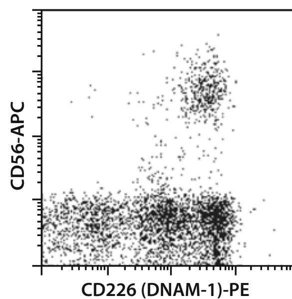
7. (Optional) If CD226 (DNAM-1)-Biotin was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL of anti-biotin antibody (Anti-Biotin-FITC, Anti-Biotin-PE, or Anti-Biotin-APC), and continue as described in steps 5 and 6.

8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

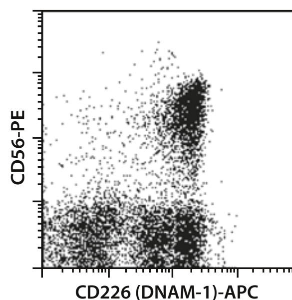
3. Examples of immunofluorescent staining with CD226 (DNAM-1) antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with CD226 (DNAM-1) antibodies conjugated to PE (a), or APC (b), as well as CD56-APC (# 130-090-843) or CD56-PE (# 130-090-755), respectively, and analyzed by flow cytometry. Cells stained with CD226 (DNAM-1)-Biotin (c) were also stained with Anti-Biotin-FITC (# 130-090-857) as well as CD56-PE. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

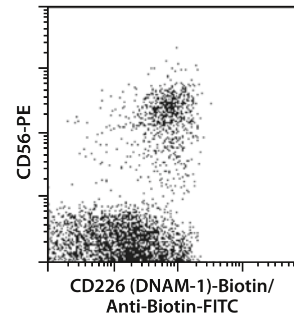
(a) Human PBMCs stained with CD226 (DNAM-1)-PE and CD56-APC.



(b) Human PBMCs stained with CD226 (DNAM-1)-APC and CD56-PE.



(c) Human PBMCs stained with CD226 (DNAM-1)-Biotin, Anti-Biotin-FITC, and CD56-PE.



4. Refereneces

1. Shibuya, A. *et al.* (1996) DNAM-1, a novel adhesion molecule involved in the cytolytic function of T lymphocytes. Immunity 4: 573–581.
2. Bottino, C. *et al.* (2003) Identification of PVR (CD155) and Nectin-2 (CD112) as cell surface ligands for the human DNAM-1 (CD226) activating molecule. J. Exp. Med. 198: 557–567.
3. Moretta, L. and Moretta, A. (2004) Unravelling natural killer cell function: triggering and inhibitory human NK receptors. EMBO J. 23: 255–259.
4. Kojima, H. *et al.* (2003) CD226 mediates platelet and megakaryocytic cell adhesion to vascular endothelial cells. J. Biol. Chem. 278: 36748–36753.
5. Castriconi, R. *et al.* (2004) Natural killer cell-mediated killing of freshly isolated neuroblastoma cells: critical role of DNAX accesory molecule-1-poliovirus receptor interaction. Cancer Res. 64: 9180–9184.
6. Pende, D. *et al.* (2005) Analysis of the receptor-ligand interactions in the natural killer-mediated lysis of freshly isolated myeloid or lymphoblastic leukemias: evidence for the involvement of the Poliovirus receptor (CD155) and Nectin-2 (CD112). Blood. 105: 2066–2073.

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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