



CD26 antibodies human

CD26-PE	130-093-440
CD26-APC	130-093-441
CD26-Biotin	130-093-442

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1. Description

Components	1 mL CD26 antibodies, human: monoclonal CD26 antibodies conjugated to R-phycoerythrin (PE), allophycocyanin (APC), or biotin.
Clone	FR10-11G9 (isotype: mouse IgG2a).
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

CD26 (DPP-IV), a type II transmembrane protein, is an ectoenzyme with prolyl oligopeptidase activity.¹ It is widely expressed in various tissues. Serum contains a soluble form of CD26. In the immune system it is expressed on thymocytes, activated T cells, B cells, NK cells, monocytes, and macrophages. It is involved in T cell activation.²

1.2 Applications

- Identification and enumeration of CD26⁺ cells by flow cytometry or fluorescence microscopy.

1.3 Recommended antibody dilution

For antibody labeling of human cells.

CD26 conjugate	PE	APC	Biotin
Flow cytometry^a			
- In general	1:11	1:11	1:11
- Formaldehyde-fixed cells	1:11	1:11	1:11

a) Given antibody dilutions are for a cell concentration of up to 10^7 cells/100 μ L of buffer.

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 CD26-Biotin.
- (Optional) CD4-FITC (# 130-092-358), CD4-PE (# 130-092-373), or CD4-APC (# 130-092-374).
- (Optional) IgG2a-PE (# 1330-091-835), or IgG2a-APC (# 130-091-836) for isotype control.
- (Optional) Propidium iodide (PI) Solution (# 130-093-233) 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 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 μ L of buffer.
4. Add 10 μ L of the CD26 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×g for 10 minutes. Aspirate supernatant completely.

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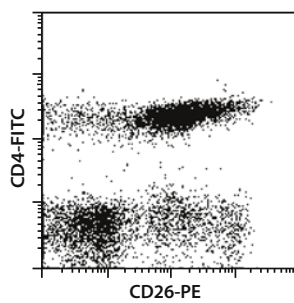


7. (Optional) If CD26-Biotin was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL of anti-biotin antibody (Anti-Biotin-FITC, Anti-Biotin-PE, or Anti-Biotin-APC), and continue as described in steps 5 and 6.
8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

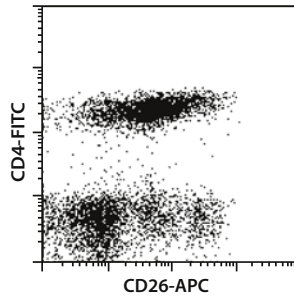
3. Examples of immunofluorescent staining with CD26 antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with CD26 antibodies conjugated to PE (a) or APC (b), and analyzed by flow cytometry. Cells stained with CD26-Biotin (c) were stained with Anti-Biotin-APC (# 130-090-856) as well as CD4-FITC. A lymphocyte gate was activated based on scatter properties of the cells. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

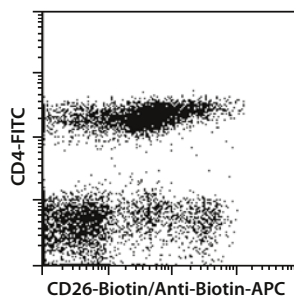
(a) Human PBMCs stained with CD26-PE.



(b) Human PBMCs stained with CD26-APC.



(c) Human PBMCs stained with CD26-Biotin, Anti-Biotin-APC, and CD4-FITC.



4. References

1. De Meester, I. *et al.* (1999) CD26, let it cut or cut it down. *Immunol. Today* 20: 367-375.
2. Reinhold, D. *et al.* (2002) The Role of Dipeptidyl Peptidase IV (DPIV) Enzymatic Activity in T Cell Activation and Autoimmunity *Biol. Chem.* 383(7-8): 1133-1138.

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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