

CD10 antibodies

human

CD10-FITC	130-093-448
CD10-PE	130-093-449
CD10-APC	130-093-450
CD10-Biotin	130-093-451

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1. Description

Components	1 mL CD10 antibodies, human: monoclonal CD10 antibodies conjugated to fluorescein isothiocyanate (FITC), R-phycoerythrin (PE), allophycocyanin (APC), or biotin.
Clone	97C5 (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

This antibody recognizes the human CD10 antigen. CD10, the human common acute lymphoblastic leukemia antigen (CALLA), is a 100 kDa cell surface molecule identical to human membrane-associated neutral endopeptidase (NEP) and also known as neprilysin or enkephalinase. Human CD10 is expressed on a wide variety of normal and neoplastic cell types from different tissues including neural and hematopoietic cells. CD10 is expressed on pre- and pro-B cells and is involved in B cell development and differentiation. The antigen is also present on mature neutrophils, T cell precursors, and some T cell leukemias/lymphomas. Furthermore, CD10 is found on neoplastic cells of several B lymphoid leukemias/lymphomas.^{1,2}

1.2 Applications

- Identification and enumeration of CD10⁺ B cells from healthy donors or patients with acute B lymphoid leukemia by flow cytometry.
- Analysis of early stages of hematopoietic differentiation.

1.3 Recommended antibody dilution

For antibody labeling of human cells.

CD10 conjugate	FITC	PE	APC	Biotin
Flow cytometry^a				
- In general	1:11	1:11	1:11	1:11
- Formaldehyde-fixed cells	1:11	1:11	1:11	1:11
- CD10 MicroBead-labeled cells	1:11	1:11	1:11	1:11

a) The indicated antibody dilutions are for up to 10⁷ cells/100 µL 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 CD10-Biotin.
- (Optional) CD19-PE (# 130-091-247) or CD19-APC (# 130-091-248). For more information about fluorochrome-conjugated antibodies see www.miltenyibiotec.com.
- (Optional) Mouse IgG1-FITC (# 130-092-213), Mouse IgG1-PE (# 130-092-212), Mouse IgG1-APC (# 130-092-214), or Mouse IgG1-Biotin (# 130-093-018) for isotype control.
- (Optional) Propidium Iodide 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 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).

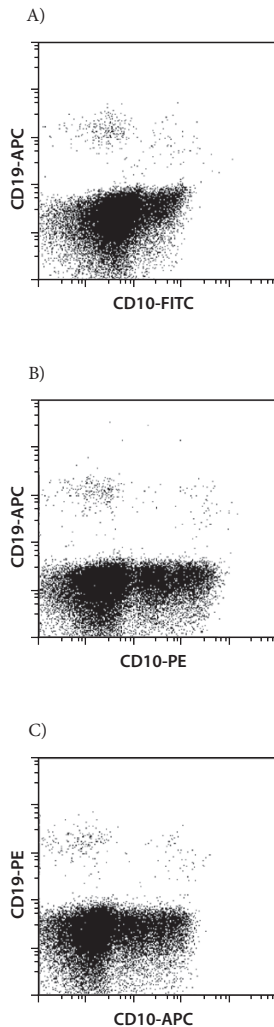
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.

3. Resuspend up to 10^7 nucleated cells per 100 μ L of buffer.
4. Add 10 μ L of the CD10 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 and centrifuge at $300\times g$ for 10 minutes. Aspirate supernatant completely.
7. (Optional) If CD10-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 CD10 antibodies

Human bone marrow cells were stained with CD10 antibodies conjugated to FITC (A), PE (B), or APC (C), as well as with CD19-PE (# 130-091-247) or CD19-APC (# 130-091-248) and analyzed by flow cytometry. Cells labeled with CD10-Biotin (D) were stained with Anti-Biotin-APC (# 130-090-856) as well as CD19-PE. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



4. References

1. Shipp, M. A. and Look, A. T. (1993) Hematopoietic differentiation antigens that are membrane-associated enzymes: cutting is the key! *Blood* 82: 1052–1070.
2. Kalled, S. L. *et al.* (1995) The distribution of CD10 (NEP 24.11, CALLA) in human and mice is similar in non-lymphoid organs but differs within the hematopoietic system: absence on murine T and B lymphoid progenitors. *Euro. J. Immunol.* 25: 677–687.

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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