



Antibodies

Index

1. Description

1.1 Background and product applications

1.2 Examples of staining concentrations

1.3 Reagent requirements

2. General protocol for immunofluorescent staining

3. Examples of immunofluorescent staining with Anti-CLA antibodies

1. Description

Clone HECA-452 (isotype: rat IgM).

Product format 1 mL Anti-CLA antibodies, human: monoclonal Anti-CLA antibodies conjugated to fluorescein-isothiocyanate (FITC), R-phycoerythrin (PE), or biotin (Biotin). The unconjugated (pure) antibody is supplied at a concentration of 100 µg/mL. Antibodies are supplied in a solution containing stabilizer and 0.05% sodium azide.

Product size 100 tests (for up to 10⁹ 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 cutaneous lymphocyte-associated antigen (CLA) is an inducible carbohydrate modification of P-selectin glycoprotein ligand 1 (PSGL-1) and functions as a ligand for E-selectin. CLA is a unique skin-homing receptor and is predominantly found on a minor subset of human T cells that infiltrate the skin. This post-translational modification of PSGL-1 is thought to serve as a mechanism to regulate tissue-specific homing of CD4⁺ and CD8⁺ memory/effector T cells from peripheral blood to the skin, which plays an essential role during many inflammatory and certain malignant skin diseases.¹⁻³

In peripheral blood, CLA is not only found on skin-homing memory/effector T cells, but is also found to be expressed on memory/effector B cells,⁴ NK cells,⁷ blood dendritic cells,⁸ and on monocytes^{1,5}. CLA is furthermore found on Langerhans cells in the skin.⁶

Product applications

- Identification and enumeration of CLA⁺ cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS[®] separations by flow cytometry or fluorescence microscopy. Human CD4⁺ or CD8⁺ T cells can be isolated by using e.g. the CD4⁺ T Cell Isolation Kit II, human (# 130-091-155), or the CD8⁺ T Cell Isolation Kit II, human (# 130-091-154), respectively. Monocytes can also be isolated from peripheral blood using the Monocyte Isolation Kit II, human (# 130-091-153).

Anti-CLA antibodies human

Anti-CLA-FITC	130-091-636
Anti-CLA-PE	130-091-635
Anti-CLA-Biotin	130-091-634
Anti-CLA pure	130-092-629

1.2 Examples of staining concentrations for human cells.

Anti-CLA conjugate	FITC	PE	Biotin
Recommended antibody dilution			
Flow cytometry^a			
- in general	1:11	1:11	1:11
- formaldehyde-fixed cells	1:11	1:11	1:11
Immunohistochemistry^b			
a) Given antibody dilutions are for a cell concentration of up to 1×10 ⁸ cells/mL buffer. b) For immunohistochemical staining, the optimal antibody dilution has to be tested.			

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 human serum albumin, human serum or fetal calf 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 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 Anti-CLA-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 Anti-CLA antibody.
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.

140-001-1111-01

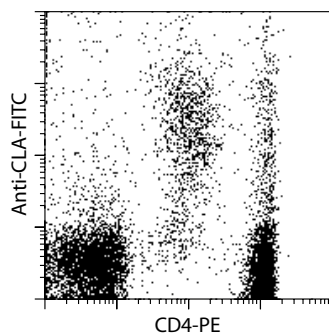


5. (Optional) If Anti-CLA-Biotin was used, resuspend the cell pellet in 100 μ L buffer, add 10 μ L Anti-Biotin antibody (Anti-Biotin-FITC #130-090-857, Anti-Biotin-PE #130-090-756, or Anti-Biotin-APC #130-090-856), and continue as described in steps 3 and 4.
6. Resuspend cell pellet in a suitable amount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

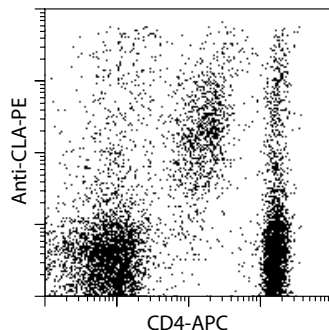
3. Examples of immunofluorescent staining with Anti-CLA antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with Anti-CLA antibodies conjugated to FITC (a), PE (b), or Biotin (c) as well as with CD4-PE (# 130-091-231) or CD4-APC (# 130-091-232). For Anti-CLA-Biotin, Anti-Biotin-APC (# 130-090-856) was used as secondary antibody. Cells were analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

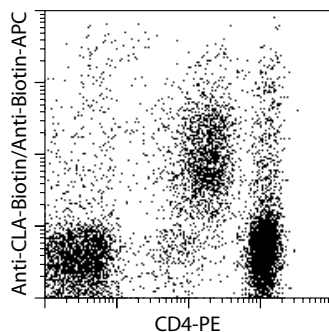
(a) Human PBMCs stained with Anti-CLA-FITC and CD4-PE.



(b) Human PBMCs stained with Anti-CLA-PE and CD4-APC.



(c) Human PBMCs stained with Anti-CLA-Biotin, Anti-Biotin-APC and CD4-PE.



4. References

1. Fulbrigge, R. C. *et al.* (1997) Cutaneous lymphocyte antigen is a specialized form of PSGL-1 expressed on skin-homing T cells. *Nature* 389: 978–981.
2. Santamaria Babi, L. F. *et al.* (1995) Migration of skin-homing T cells across cytokine-activated human endothelial cell layers involves interaction of the cutaneous lymphocyte-associated antigen (CLA), the very late antigen-4 (VLA-4), and the lymphocyte function-associated antigen-1 (LFA-1). *J. Immunol.* 154: 1543–1550.
3. Takahashi, R. *et al.* (2003) In vitro differentiation from naive to mature E-selectin binding CD4 T cells: acquisition of skin-homing properties occurs independently of cutaneous lymphocyte antigen expression. *J. Immunol.* 171: 5769–5777.
4. Yoshino, T. *et al.* (1999) Cutaneous lymphocyte antigen is expressed on memory/effector B cells in the peripheral blood and monocytoid B cells in the lymphoid tissues. *Immunology* 98: 9–15.
5. Bos, J. D. *et al.* (1993) Skin-homing T lymphocytes: detection of cutaneous lymphocyte-associated antigen (CLA) by HECA.452 in normal human skin. *Arch. Dermatol. Res.* 285: 179–183.
6. Strunk, D. *et al.* (1997) A skin homing molecule defines the Langerhans cell progenitor in human peripheral blood. *J. Exp. Med.* 185: 1131–1136.
7. Tsuchiyama, J. *et al.* (2002) Induction and characterization of cutaneous lymphocyte antigen on natural killer cells. *Br. J. Haematol.* 118(2): 654–662.
8. Dzionek, A. *et al.* (2000) BDCA-2, BDCA-3, and BDCA-4: three markers for distinct subsets of dendritic cells in human peripheral blood. *J. Immunol.* 165(11): 6037–6046.

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.

Warranty

The products sold hereunder are warranted only to be free from defects in workmanship and material at the time of delivery to the customer. Miltenyi Biotec GmbH makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the technical specifications of the products. Miltenyi Biotec GmbH's liability is limited to either replacement of the products or refund of the purchase price. Miltenyi Biotec GmbH is not liable for any property damage, personal injury or economic loss caused by the product.

MACS is a registered trademark of Miltenyi Biotec GmbH.

© 2006 Miltenyi Biotec GmbH. Printed in Germany.