



CD57 antibodies human

CD57-FITC	130-092-174
CD57-PE	130-092-139
CD57-APC	130-092-141
CD57-Biotin	130-092-620
CD57-VioBlue®	130-096-530

Contents

1. Description
 - 1.1 Background information
 - 1.2 Applications
 - 1.3 Recommended antibody dilution
 - 1.4 Reagent requirements
2. General protocol for immunofluorescent staining
3. Examples of immunofluorescent staining with CD57 antibodies
4. References

1. Description

Components	1 mL CD57 antibodies, human: monoclonal CD57 antibodies conjugated to fluorescein isothiocyanate (FITC), R-phycoerythrin (PE), allophycocyanin (APC), biotin, or VioBlue®.
Clone	TB03 (isotype: mouse IgM).
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

CD57, also known as HNK-1 or Leu-7, is an antigenic oligosaccharide moiety detected on extracellular proteins of certain cell types. In blood, CD57 is found on 15–20% of mononuclear cells¹, including subsets of NK and T cells, though not on erythrocytes, monocytes, granulocytes, or platelets. Also, CD57 expression can be found on a variety of neural cell types.^{1,2}

CD57 has been shown to be expressed on late stage effector CD8⁺ T cells³. The frequency of CD3⁺CD57⁺ lymphocytes is raised in a variety of diseases, for example, in the blood and synovia of sufferers of rheumatoid arthritis⁴. CD57 expression is also increased on chronically activated CD8⁺ T cells in persistent viral infections, such as HIV.⁵

1.2 Applications

- Identification and enumeration of CD57⁺ cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy. CD57⁺ human cells can be isolated using the CD57 MicroBeads, human (# 130-092-073).

1.3 Recommended antibody dilution

For antibody labeling of human cells.

CD57 conjugate	FITC	PE	APC	Biotin	VioBlue
Flow cytometry^a					
- In general	1:11	1:11	1:11	1:11	1:11
- Formaldehyde-fixed cells ^b	1:11	1:11	1:11	1:11	1:11
- CD57 MicroBead-labeled cells	1:11	1:11	1:11	1:11	1:11

a) Given antibody dilutions are for a cell concentration of up to 10⁷ 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 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 CD57-Biotin.
- (Optional) CD8-FITC (# 130-080-601) or CD56-PE (# 130-090-755).
- (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 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 10⁷ nucleated cells per 100 µL of buffer.
4. Add 10 µL of the CD57 antibody.

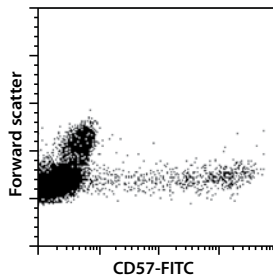


5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).
 - ▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.
6. Wash cells by adding 1–2 mL of buffer per 10⁷ cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. (Optional) If CD57-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.

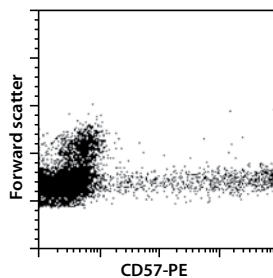
3. Examples of immunofluorescent staining with CD57 antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with CD57 antibodies conjugated to FITC (a), PE (b), APC (c), or VioBlue (d) and analyzed by flow cytometry. Cells stained with CD57-Biotin (e) were stained with Anti-Biotin-PE (# 130-090-756). Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

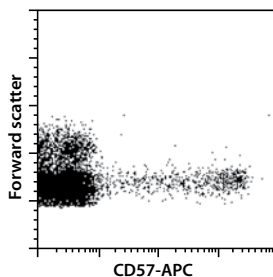
(a) Human PBMCs stained with CD57-FITC.



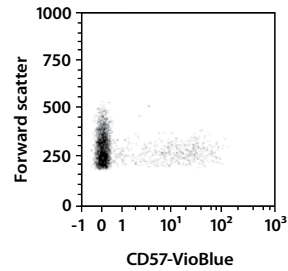
(b) Human PBMCs stained with CD57-PE.



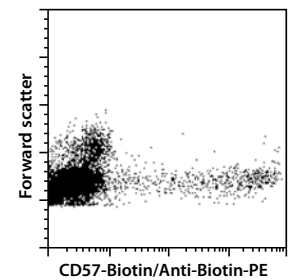
(c) Human PBMCs stained with CD57-APC.



(d) Human PBMCs stained with CD57-VioBlue.



(e) Human PBMCs stained with CD57-Biotin and Anti-Biotin-PE.



4. References

1. Mechtersheimer, G. *et al.* (1991) Expression of the natural killer cell-associated antigens CD56 and CD57 in human neural and striated muscle cells and in their tumors. *Cancer Res.* 51: 1300–1307.
2. Ong, E. *et al.* (2002) Biosynthesis of HNK-1 glycans on O-linked oligosaccharides attached to the neural cell adhesion molecule (NCAM): the requirement for core 2 beta 1,6-N-acetylglucosaminyltransferase and the muscle-specific domain in NCAM. *J. Biol. Chem.* 277: 18182–18190.
3. Brenchley J. M. *et al.* (2003) Expression of CD57 defines replicative senescence and antigen-induced apoptotic death of CD8⁺ T cells. 101: 2711–2720.
4. Dupuy de'Angeac, A. *et al.* (1993) Increased percentage of CD3⁺, CD57⁺ lymphocytes in patients with rheumatoid arthritis. Correlation with duration of disease. *Arthritis Rheum.* 36: 608–612.
5. Palmer, B. E. *et al.* (2005) Functional and phenotypic characterization of CD57⁺CD4⁺ T cells and their association with HIV-1-induced T cell dysfunction. *J. Immunol.* 175: 8415–8423.

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.

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.

autoMACS, MACS, and VioBlue are registered trademarks of Miltenyi Biotec GmbH.

Copyright © 2011 Miltenyi Biotec GmbH. All rights reserved.