

CD161 antibodies human

CD161-FITC	130-092-907
CD161-PE	130-092-677
CD161-APC	130-092-678
CD161-Biotin	130-092-906
CD161 pure	130-092-676

Index

1. Description
 - 1.1 Background and product applications
 - 1.2 Recommended antibody dilution
 - 1.3 Reagent requirements
2. General protocol for immunofluorescent staining
3. Examples of immunofluorescent staining with CD161 antibodies
4. References

1. Description

Clone	191B8 (isotype: mouse IgG2a).
Product format	1 mL CD161 antibodies, human: monoclonal CD161 antibodies conjugated to fluorescein isothiocyanate (FITC), R-phycoerythrin (PE), allophycocyanin (APC), or 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 or up to 10 ⁹ total cells.
Storage	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Background and product applications

CD161, also known as NKR-P1A, is a C-type lectin membrane glycoprotein expressed as an 80 kDa disulphide-linked homodimer. It is expressed on most natural killer (NK) cells and subsets of T cells, including NKT cells. In peripheral blood CD161 is preferentially expressed on T cells of a memory phenotype, but it can also be found on subsets of thymocytes, on fetal liver T cells, and on human monocytes and dendritic cells. CD161 has been implicated in the triggering of NK-mediated cytotoxicity, contributing to target cell recognition by NK cells.^{1–4}

Product applications

- Identification and enumeration of CD161⁺ cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® Separations of human NK and NKT cells by flow cytometry or fluorescence microscopy. Human NK cells can be isolated by using the NK Cell Isolation Kit, human (# 130-092-657). Human NKT cells can be isolated by using the CD3⁺CD56⁺ NKT Cell Isolation Kit, human (# 130-093-064).

1.2 Recommended antibody dilution

For antibody labeling of human cells.

CD161 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

a) Given antibody dilutions are for a cell concentration of up to 10⁷ cells/100 µL of buffer.

1.3 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 (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 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 CD161-Biotin.
- (Optional) CD3-FITC, human (# 130-080-401), CD3-PE (# 130-091-374), CD3-APC (# 130-091-373), CD56-PE (# 130-090-755), or CD56-APC, human (# 130-090-843).
- (Optional) Propidium iodide (PI) or 7-AAD for flow cytometric exclusion of dead cells without 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).

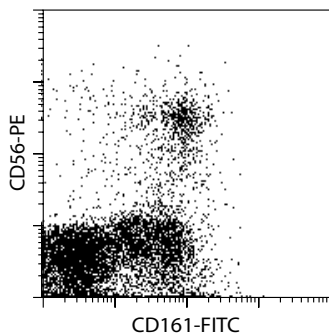
1. Resuspend up to 10⁷ nucleated cells per 100 µL of buffer.
2. Add 10 µL of the CD161 antibody.
▲ Note: See table for exceptions.
3. Mix well and refrigerate for 10 minutes in the dark (4–8 °C).
▲ Note: Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times may 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. Aspirate supernatant completely.

5. (Optional) If CD161-Biotin was used, resuspend the cell pellet in 100 μ L of buffer, add 10 μ L of 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.

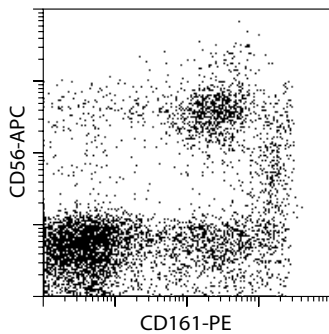
3. Examples of immunofluorescent staining with CD161 antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with CD161 antibodies conjugated to FITC (a), PE (b), or APC (c), as well as CD56-PE or CD56-APC, and analyzed by flow cytometry. Cells stained with CD161-Biotin (d) were stained with Anti-Biotin-APC (# 130-090-856) as well as CD56-PE. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence. A lymphocyte gate was set.

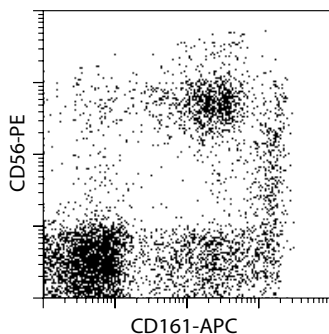
(a) Human PBMCs stained with CD161-FITC and CD56-PE.



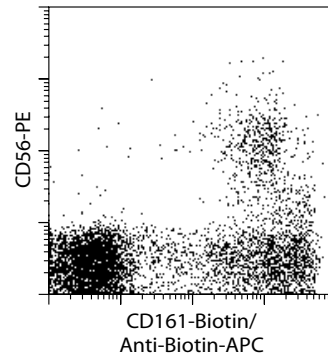
(b) Human PBMCs stained with CD161-PE and CD56-APC.



(c) Human PBMCs stained with CD161-APC and CD56-PE.



(d) Human PBMCs stained with CD161-Biotin, Anti-Biotin-APC, and CD56-PE.



4. References

1. Lanier, L.L. *et al.* (1994) Human NKR-P1A. A disulfide-linked homodimer of the C-type lectin superfamily expressed by a subset of NK and T lymphocytes. *J. Immunol.* 153: 2417–2428.
2. Poggi, A. *et al.* (2002) Transendothelial migratory pathways of $V\delta 1^+ TCR\gamma\delta^+$ and $V\delta 2^+ TCR\gamma\delta^+$ T lymphocytes from healthy donors and multiple sclerosis patients: involvement of phosphatidylinositol 3 kinase and calcium calmodulin-dependent kinase II. *J. Immunol.* 168: 6071–6077.
3. Takahashi, T. *et al.* (2006) Expression of CD161 (NKR-P1A) defines subsets of human CD4 and CD8 T cells with different functional activities. *J. Immunol.* 176: 211–216.
4. Pozo, D. *et al.* (2006) CD161 (human NKR-P1A) signaling in NK cells involves the activation of acid sphingomyelinase. *J. Immunol.* 176: 2397–2406.

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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