CD56 antibodies, human

For research use only

One test corresponds to labeling of up to 10^7 cells in a total volume of 100 µL.

Product	Content	Order no.
CD56-VioBright FITC[1]	for 30 tests	130-104-997
CD56-VioBright FITC[1]	for 100 tests	130-104-944
CD56-PE[1]	for 30 tests	130-098-137
CD56-PE[1]	for 100 tests	130-090-755
CD56-APC[1]	for 30 tests	130-098-135
CD56-APC[1]	for 100 tests	130-090-843
CD56-PE-Vio770[1]	for 30 tests	130-098-132
CD56-PE-Vio770[1]	for 100 tests	130-096-831
CD56-Biotin[1]	for 100 tests	130-098-557
CD56 pure[1]	50 µg in 1 mL	130-090-955

[1]Not recommended for cells that are labeled with MACS MicroBeads using the same antigen.

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.

Technical data and background information

Antigen	CD56
Clone	AF12-7H3
Isotype	mouse IgG1κ
Isotype control	Mouse IgG1 – isotype control antibodies
Alternative names of antigen	NCAM1, MSK39, NCAM, Leu-19, NKH-1
Molecular mass of antigen [kDa]	92
Cross-reactivity	rhesus monkey (*Macaca mulatta*), cynomolgus monkey (*Macaca fascicularis*)
Distribution of antigen	brain, cardiac muscle, leukemia cells, liver, lung, NK cells, ES and iPS cells, skeletal muscle, smooth muscle, spleen, T cells
Product format	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.
Fixation	Cells should be stained prior to fixation, if formaldehyde is used as a fixative.
Storage	Store protected from light at 2–8 °C. Do not freeze.

Clone AF12-7H3 recognizes the human CD56 antigen, a glycoprotein of the Ig-superfamily also known as neural cell adhesion molecule (NCAM) which is expressed in blood on practically all resting and activated NK cells and on a minor subset of $CD3^+$ T cells. CD56 is reported to be expressed on rhesus monkey monocytes but not on NK cells.[1]
CD56 is also expressed in brain (cerebellum and cortex) and at neuromuscular junctions. Certain large granular lymphocyte (LGL) leukemias, small-cell lung carcinomas, neuronal-derived tumors, myelomas, and myeloid leukemias also express CD56.
The monoclonal antibody AF12-7H3 recognizes an epitope distinct from those recognized by the CD56-specific mAbs NCAM16.2 and B159.

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). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.
- (Optional) FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor–mediated antibody labeling.
- (Optional) Fluorochrome-conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with biotinylated antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) 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.

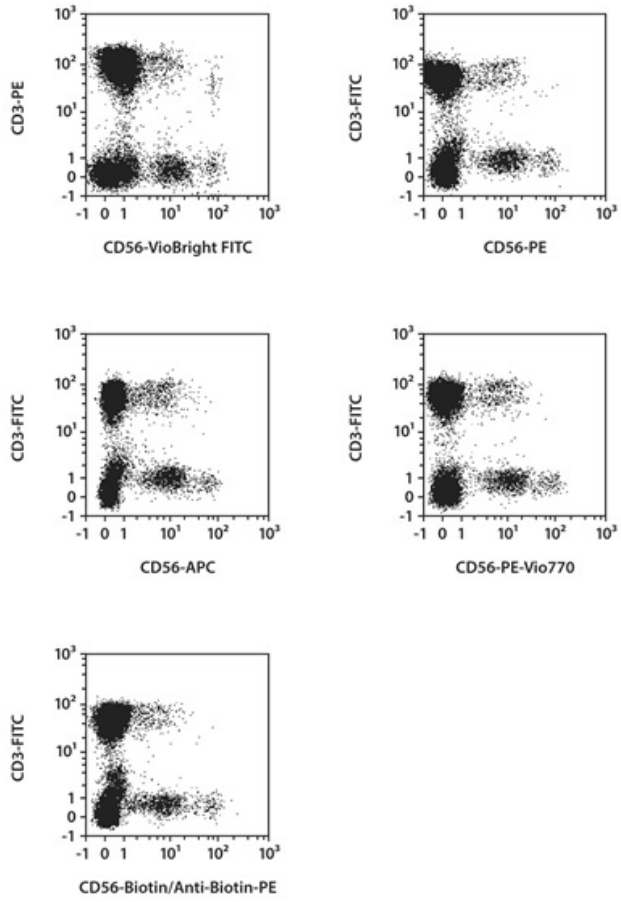
Protocol for cell surface staining

- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:11 for up to 10^7 cells/100 µL of buffer.
- 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).

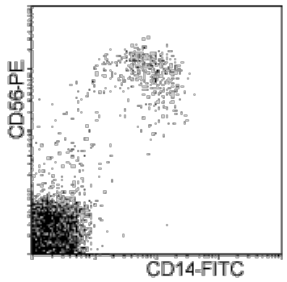
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^7 nucleated cells per 100 µL of buffer.
4. Add 10 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   Note: Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.
6. Wash cells by adding 1−2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. (Optional) If biotinylated antibody was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL of fluorochrome-conjugated anti-biotin antibody, 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.

Examples of immunofluorescent staining

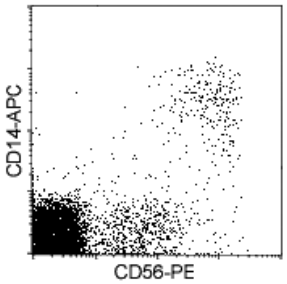
Human peripheral blood mononuclear cells (PBMCs) were stained with CD56 antibodies as well as with CD3 antibodies and analyzed by flow cytometry using the MACSQuant® Analyzer. The Tandem Signal Enhancer has been used to increase binding specificity of tandem-dye–conjugated antibodies. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence or 4',6-diamidino-2-phenylindole (DAPI) fluorescence, as in the case of tandem conjugates.



Rhesus monkey PBMCs were stained with CD56-PE and CD14-FITC and analyzed by flow cytometry.



Cynomolgus monkey PBMCs were stained with CD56-PE and CD14-APC and analyzed by flow cytometry.



References

1. **Carter, D. L. *et al.*** (1999) CD56 identifies monocytes and not natural killer cells in *Rhesus macaques*. Cytometry 37: 41–50.
2. **Meng, J. *et al.*** (2011) Contribution of human muscle-derived cells to skeletal muscle regeneration in dystrophic host mice. PLoS One 6(3): e17454.

3. **Stockholm, D. *et al.*** (2011) Bistable cell fate specification as a result of stochastic fluctuations and collective spatial cell behaviour. PLoS One 5(12): e14441.

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.

Miltenyi Biotec GmbH | Friedrich-Ebert-Straße 68 | 51429 Bergisch Gladbach | Germany | Phone +49 2204 8306-0 | Fax +49 2204 85197 | macs@miltenyibiotec.de | **www.miltenyibiotec.com**
Miltenyi Biotec provides products and services worldwide. Visit **www.miltenyibiotec.com/local** to find your nearest Miltenyi Biotec contact.