Miltenyi Biotec

CD56 antibodies
human

Contents

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.

1. Description

This product is for research use only.

Components Monoclonal CD56 antibodies, human conjugated to:

Conjugate	Order no. 1 mL (100 tests)	Order no. 300 µL (30 tests)
VioBright™ FITC*	130-104-944	130-104-997
PE*	130-090-755	130-098-137
APC*	130-090-843	130-098-135
PE-Vio770™*	130-096-831	130-098-132
Biotin*	130-098-557	–
pure*	130-090-955	–

Clone AF12-7H3 (isotype: mouse IgG1).

Capacity 1 mL: 100 tests or up to 10^9 total cells

300 µL: 30 tests or up to 3×10^8 total cells.

The unconjugated (pure) antibody is supplied at a concentration of 100 µg/mL.

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.

* Not recommended for cells that are labeled with MACS MicroBeads using the same antigen.

Cross-reactivity: The CD56 antibody has been reported to react with

- rhesus monkey (*Macaca mulatta*) cells
- cynomolgus monkey (*Macaca fascicularis*) cells

1.1 Background information

- Antigen: CD56
- Synonym: Leu-19; NCAM; NKH-1
- Expression patterns: 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.

1.2 Applications

- Identification and enumeration of $CD56^+$ cells by flow cytometry.

1.3 Recommended antibody dilution

The recommended antibody dilution for all CD56 conjugates is **1:11 for up to 10^7 cells/100 µL** of buffer for labeling of cells and subsequent analysis by flow cytometry.

Cells should be stained prior to fixation, if formaldehyde is used as a fixative.

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). 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) Tandem Signal Enhancer, human (# 130-099-888) to reduce non-specific binding of tandem dye–conjugated antibodies to human cells, especially to monocytes.
- (Optional) Conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with CD56-Biotin.
- (Optional) For antibodies for additional staining or for isotype

140-002-184.05

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control, refer to www.miltenyibiotec.com/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.

2. General protocol for immunofluorescent staining

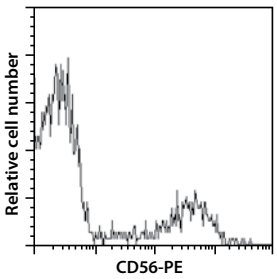
Volumes given below are for **up to 10^7** nucleated cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^7 nucleated cells, use twice the volume of all indicated 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 CD56 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 CD56-Biotin was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL of 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.

3. Example of immunofluorescent staining with CD56 antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with CD56 antibodies conjugated to PE and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



For more examples please refer to the respective product page at www.miltenyibiotec.com/antibodies.

4. References

1. Carter, D. L. *et al.* (1999) CD56 identifies monocytes and not natural killer cells in *Rhesus macaques*. Cytometry 37: 41–50.

Refer to www.miltenyibiotec.com for all data sheets and protocols.

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