Miltenyi Biotec

CD25 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 CD25 antibodies, human conjugated to:

Conjugate	Order no. 1 mL (100 tests)	Order no. 300 µL (30 tests)
VioBright™ FITC	130-104-274	130-104-323
PE	130-091-024	130-098-211
APC	130-092-858	130-098-213
Biotin	130-091-235	130-098-114

Clone 4E3 (isotype: mouse IgG2b).

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

300 µL: 30 tests or up to 3×10^8 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.

Cross-reactivity: The CD8 (BW135/80) antibody has been tested to react with

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

1.1 Background information

- Antigen: CD25
- Synonym: IL-2Rα; p55; Tac
- Expression patterns: The CD25 antibody recognizes the human CD25 antigen, a 28 kDa glycoprotein also known as the low-affinity interleukin-2 receptor alpha chain (IL-2Rα). CD25 is expressed on activated T and B cells, on macrophages, and on a subset of non-activated $CD4^+$ regulatory T cells. The CD25 antigen contains three epitope regions called A, B, and C. This CD25 antibody recognizes epitope region B.

1.2 Applications

- Identification and enumeration of $CD25^+$ cells by flow cytometry.

1.3 Recommended antibody dilution

The recommended antibody dilution for all CD25 conjugates is **1:11 for up to 10^7 cells/100 µL** of buffer for labeling of cells and subsequent analysis by flow cytometry.

For CD25 MicroBead-labeled cells the dilution is **1:6 for up to 10^7 cells/100 µL** of buffer.

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) Conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with CD25-Biotin.
- (Optional) For antibodies for additional staining or for isotype 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).

140-002-037.05

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

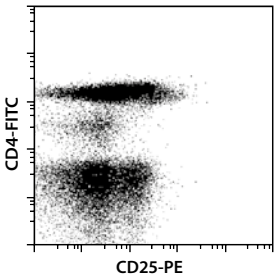
Miltenyi Biotec Inc.
2303 Lindbergh Street, Auburn, CA 95602, USA
Phone 800 FOR MACS, +1 530 888 8871, Fax +1 877 591 1060
macs@miltenyibiotec.com

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 CD25 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 CD25-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 CD25 antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with CD25 antibodies conjugated to PE as well as with CD4 (VIT4)-FITC and analyzed by flow cytometry using the MACSQuant® Analyzer. 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. Sakaguchi, S. *et al*. (1995) Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J. Immunol. 155: 1151–1164.
2. Shevach, E. M. (2001) Certified professionals: $CD4^+CD25^+$ suppressor T cells. J. Exp. Med. 193: F41–F46.
3. Maloy, K. J. and Powrie, F. (2001) Regulatory T cells in the control of immune pathology. Nature Immunol. 2: 816–822.

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

Warranty