Miltenyi Biotec

CD8 (BW135/80) 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 CD8 (BW135/80) antibodies, human conjugated to:

Conjugate	Order no. 1 mL (100 tests)	Order no. 300 µL (30 tests)
FITC	130-080-601	130-098-059
VioBright™ FITC	130-104-519	130-104-568
PE	130-091-084	130-098-075
APC	130-091-076	130-098-078
VioBlue®	130-094-152	130-098-066
VioGreen™	130-096-902	130-098-062
PerCP	130-094-972	130-098-057
PE-Vio770™	130-096-556	130-098-060
APC-Vio770™	130-096-561	130-098-065
PerCP-Vio700™	130-097-911	–
Biotin	130-098-556	130-098-555

Clone BW135/80 (isotype: mouse IgG2a).

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: CD8
- Synonym: CD8; Leu-2; Ly-2; T8
- Expression patterns: The CD8 antibody recognizes the human CD8 antigen which is strongly expressed on human cytotoxic T cells and thymocytes, and is also expressed on a subset of NK cells. The CD8 antigen is a disulfide-linked dimer that exists either as a CD8α homodimer or as a CD8α/β heterodimer. CD8 acts as a coreceptor for the T cell receptor and binds to the MHC Class I molecule. The CD8 antibody recognizes the α-subunit of the antigen.

1.2 Applications

- Identification and enumeration of $CD8^+$ cells by flow cytometry.

1.3 Recommended antibody dilution

The recommended antibody dilution for all CD8 (BW135/80) 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 CD8-Biotin.
- (Optional) For antibodies for additional staining or for isotype control, refer to www.miltenyibiotec.com/antibodies.

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- (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

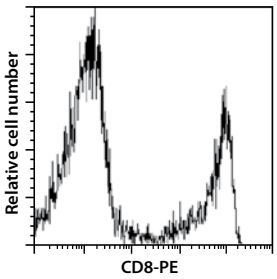
Volumes given below are for **up to 10^7** nucleated cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^7 nucleated cells, use twice the volume of all indicated 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 CD8 (BW135/80) 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 CD8 (BW135/80)-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 CD8 (BW135/80) antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with CD8 (BW135/80) 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. Jonker, M. *et al.* (1989) Reactivity of mAb specific for human CD markers with rhesus monkey leucocytes. Oxford, Oxford University Press (Leukocyte Typing IV): 1058–1063.

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

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.

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