Miltenyi Biotec

CD3 antibodies
human

Contents

1. Description

Components 1 mL monoclonal CD3 antibodies, human conjugated to various dyes.

FITC	130-080-401
PE	130-091-374
APC	130-091-373
VioBlue®	130-094-363
VioGreen™	130-096-910
PerCP	130-094-965
PE-Vio770™	130-096-749
APC-Vio770	130-096-610
PerCP-Vio700	130-097-582
Biotin	130-098-612

Clone BW264/56 (isotype: mouse IgG2a).

Capacity 100 tests or up to 10^9 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.

1.1 Background information

The CD3 antigen is present on mature human T cells, thymocytes, and NKT cells. It is associated with the T cell receptor (TCR) and is responsible for the signal transduction of the TCR. The CD3 antigen is a complex of five invariable chains: γ, δ, ε, ζ, and η. The CD3 antibody recognizes all T cells, i.e., it reacts with 70–80% of human peripheral blood lymphocytes and with 65–85% of thymocytes. The epitope recognized by the antibody is located on the ε-chain of the CD3 complex.

1.2 Applications

- Identification and enumeration of $CD3^+$ cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy. Human T cells can be isolated by using, for example, CD3 MicroBeads, human (# 130-050-101), Pan T Cell Isolation Kit, human (# 130-096-535), or Whole Blood CD3 MicroBeads, human (# 130-090-874).

1.3 Recommended antibody dilution

The recommended antibody dilution for all CD3 conjugates is **1:11 for up to 10^7 cells/100 µL** of buffer for labeling of cells and analysis by flow cytometry. For CD3 MicroBead-labeled cells use the same dilution. CD3-VioGreen and CD3-APC-Vio770 for staining of cells labeled with CD3 MicroBeads are not recommended.

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). BSA can be replaced by other proteins such as human serum albumin, human serum, or fetal bovine serum (FBS). 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) Anti-Biotin antibodies conjugated to, e.g., PE (# 130-090-756) as secondary antibody reagent in combination with CD3-Biotin.
- (Optional) Mouse IgG2a isotype control antibodies conjugated to, e.g., VioBlue (# 130-094-671). For more information about isotype control antibodies refer to www. miltenyibiotec.com.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD 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.

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2. Resuspend up to 10^7 nucleated cells per 100 µL of buffer.

   ▲ **Note:** (Optional) If FcR Blocking Reagent, human is being used, resuspend 10^7 nucleated cells in 90 µL of buffer and add 10 µL of FcR Blocking Reagent, human directly before addition of the antibody.

3. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
4. Resuspend up to 10^7 nucleated cells per 100 µL of buffer.
5. Add 10 µL of the CD3 antibody.
6. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).

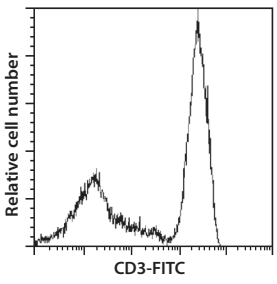
   ▲ **Note:** Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.

7. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
8. (Optional) If CD3-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.
9. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

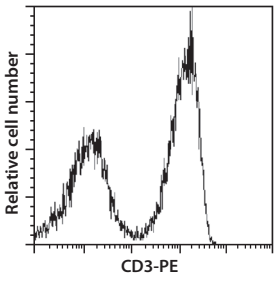
3. Examples of immunofluorescent staining with CD3 antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with CD3 antibodies conjugated to FITC (A), PE (B), APC (C), VioBlue (D), VioGreen (E), PerCP (F), PE-Vio770 (G), APC-Vio770 (H), or PerCP-Vio700 (I) and analyzed by flow cytometry using the MACSQuant® Analyzer. Cells labeled with CD3-Biotin (J) were stained with Anti-Biotin-PE (# 130-090-756). Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

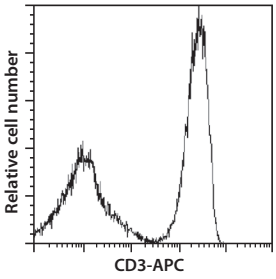
A)



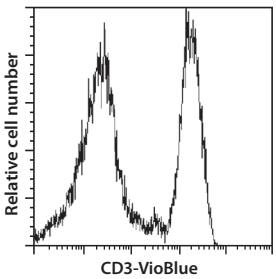
B)



C)



D)



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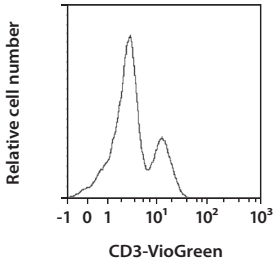

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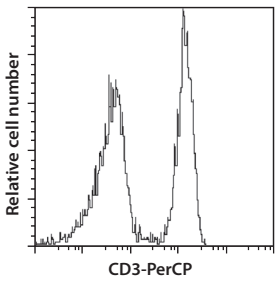

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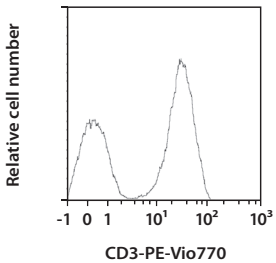

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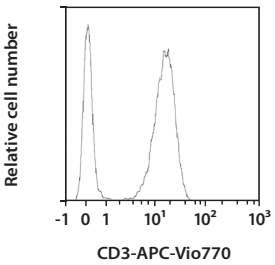
H)



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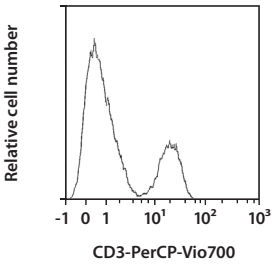
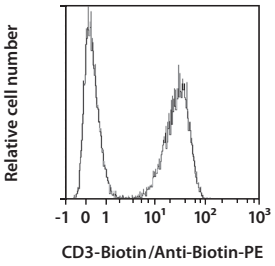


J)



All protocols and data sheets are available at **www.miltenyibiotec.com**.

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.

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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