Miltenyi Biotec

CD4 (VIT4) antibodies
human

Contents

1. Description

Components	1 mL CD4 (VIT4) antibodies, human conjugated to various dyes.	
	FITC	130-092-358
	PE	130-092-373
	APC	130-092-374
	VioBlue®	130-094-153
	VioGreen™	130-096-900
	PerCP	130-094-963
	PE-Vio770™	130-096-552
	APC-Vio770	130-096-652
Clone	VIT4 (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

CD4 is a type I transmembrane glycoprotein involved in the recognition of MHC class II/peptide complexes by the TCR heterodimers. CD4 is highly expressed on T helper cells and at a lower level on monocytes and dendritic cells. The CD4 molecule is the receptor for the human immunodeficiency virus.
The CD4 (VIT4) antibody recognizes most thymocytes and about 65% of all peripheral blood T cells.

1.2 Applications

- Identification and enumeration of $CD4^+$ cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy. Human T helper cells can be isolated by using, for example, CD4 MicroBeads, human (# 130-045-101) or the $CD4^+$ T Cell Isolation Kit, human (# 130-096-533).

1.3 Recommended antibody dilution

The recommended antibody dilution for all CD4 (VIT4) conjugates is **1:11 for up to 10^7 cells/100 µL** of buffer for labeling of cells and analysis by flow cytometry. For CD4 MicroBead-labeled cells use the same dilution.

The CD4 (VIT4)-VioBlue antibody is not suited for staining of formaldehyde-fixed cells.
All other conjugates are suited for staining of formaldehyde-fixed cells.
For optimal results, cells must be stained prior to fixation with formaldehyde.

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) 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.
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.

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4. Add 10 µL of the CD4 (VIT4) antibody.

   ▲ **Note:** Refer to section 1.3 for exceptions.

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.

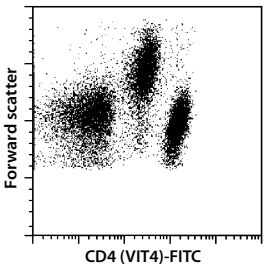
6. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.

7. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

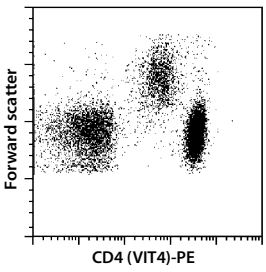
3. Examples of immunofluorescent staining with CD4 (VIT4) antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with CD4 (VIT4) antibodies conjugated to FITC (A), PE (B), APC (C), VioBlue (D), VioGreen (E), PerCP (F), PE-Vio770 (G), or APC-Vio770 (H) 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.

A)



B)



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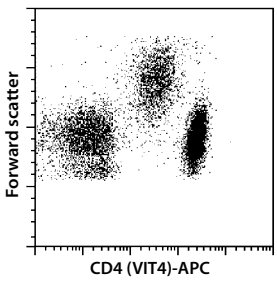

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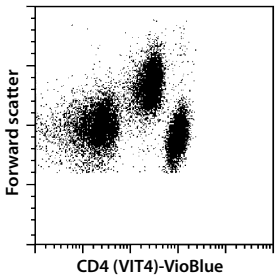

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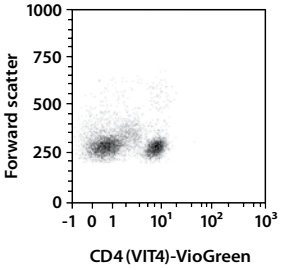

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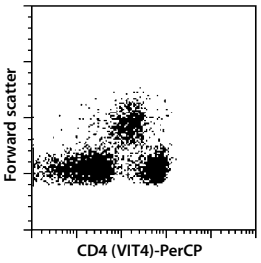

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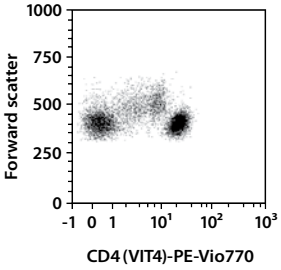

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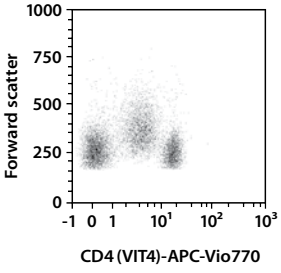

All protocols and data sheets are available at **www.miltenyibiotec.com**.

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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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