

Contents

1. Description
 - 1.1 Background information
 - 1.2 Applications
 - 1.3 Recommended antibody dilution
 - 1.4 Reagent requirements
2. General protocol for immunofluorescent staining
3. Examples of immunofluorescent staining with CD4 antibodies
4. References

1. Description

Components	1 mL monoclonal CD4 antibodies, human conjugated to various dyes.								
	<table border="0"> <tr> <td>VioBlue®</td> <td>130-097-333</td> </tr> <tr> <td>FITC</td> <td>130-080-501</td> </tr> <tr> <td>PE</td> <td>130-091-231</td> </tr> <tr> <td>APC</td> <td>130-091-232</td> </tr> </table>	VioBlue®	130-097-333	FITC	130-080-501	PE	130-091-231	APC	130-091-232
VioBlue®	130-097-333								
FITC	130-080-501								
PE	130-091-231								
APC	130-091-232								
Clone	MT466 (isotype: mouse IgG1).								
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.								

Cross-reactivity: The CD4 antibody has been tested [reported] to react with rhesus monkey (*Macaca mulatta*) and cynomolgus monkey (*Macaca fascicularis*) cells.

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 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 II, human (# 130-091-155). Rhesus monkey (*Macaca mulatta*) CD4⁺ cells can be isolated by using CD4 MicroBeads, non-human primate (# 130-091-102).

1.3 Recommended antibody dilution

The recommended antibody dilution for all CD4 conjugates is **1:11 for up to 10⁷ cells/100 µL** of buffer for labeling of cells and analysis by flow cytometry.

Except the FITC conjugate is the antibody suited for staining of formaldehyd-fixed cells. Staining intensity might be reduced on fixed cells.

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 Ca²⁺ or Mg²⁺ are not recommended for use.
- (Optional) FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor-mediated antibody labeling.
- (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

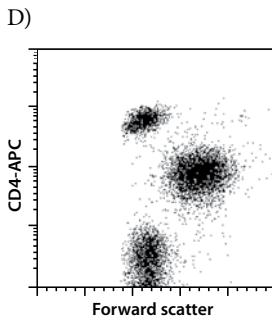
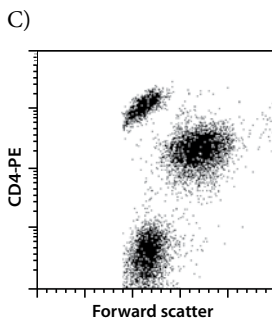
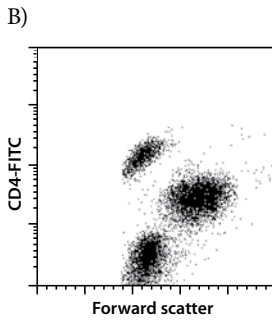
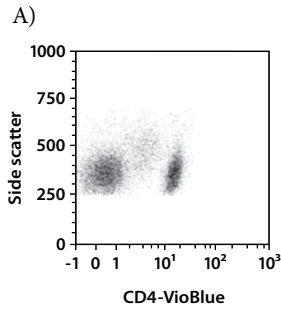
▲ Volumes given below are for up to 10⁷ nucleated cells. When working with fewer than 10⁷ cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10⁷ nucleated cells, use twice the volume of all indicated 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 10⁷ nucleated cells per 100 µL of buffer.
4. Add 10 µL of the CD4 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 antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with CD4 antibodies conjugated to VioBlue (A), FITC (B), PE (C), or APC (D) 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.



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.

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