

### 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 CD8 antibodies
4. Reference

## 1. Description

<b>Components</b>	1 mL monoclonal CD8 antibodies, human conjugated to various dyes.
	FITC 130-080-601
	PE 130-091-084
	APC 130-091-076
	VioBlue® 130-094-152
	VioGreen™ 130-096-902
	PerCP 130-094-972
	PE-Vio770™ 130-096-556
	APC-Vio770 130-096-561
<b>Clone</b>	BW135/80 (isotype: mouse IgG2a).
<b>Capacity</b>	100 tests or up to 10 <sup>9</sup> total cells.
<b>Product format</b>	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.
<b>Storage</b>	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

Cross-reactivity: The CD8 antibody is tested to react with rhesus monkey (*Macaca mulatta*)<sup>1</sup> and cynomolgus monkey (*Macaca fascicularis*) cells.

### 1.1 Background information

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 CD8 $\alpha$  homodimer or as a CD8 $\alpha$ / $\beta$  heterodimer. CD8 acts as a co-receptor for the T cell receptor and binds to the MHC Class I molecule. The CD8 antibody recognizes the  $\alpha$ -subunit of the antigen.

### 1.2 Applications

- Identification and enumeration of human cytotoxic T cells by flow cytometry or fluorescence microscopy.

- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy. Human CD8<sup>+</sup> can be isolated by using, for example, CD8 MicroBeads (# 130-045-201), the CD8<sup>+</sup> T Cell Isolation Kit (# 130-096-495), or the CD8 MultiSort Kit (# 130-055-201).

### 1.3 Recommended antibody dilution

The recommended antibody dilution for all CD8 conjugates is **1:11 for up to 10<sup>7</sup> cells/100  $\mu$ L** of buffer for labeling of cells and analysis by flow cytometry. For CD8 MicroBead-labeled cells use the same dilution.

The antibody is 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<sup>2+</sup> or Mg<sup>2+</sup> are not recommended for use.
- 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](http://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

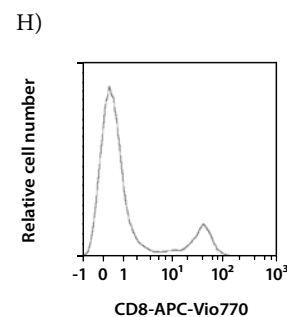
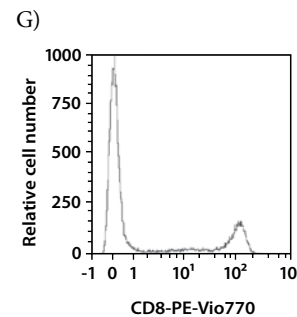
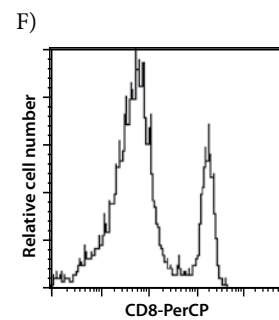
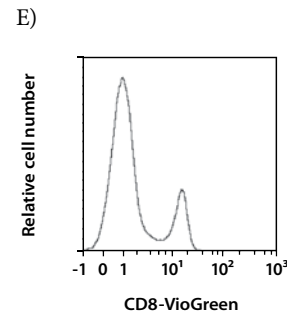
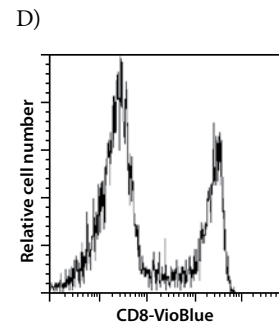
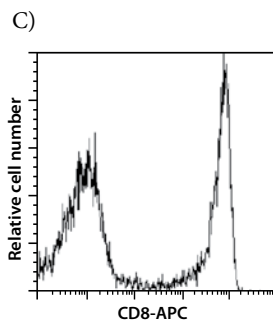
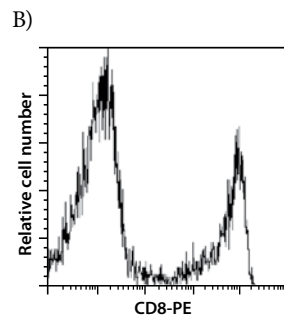
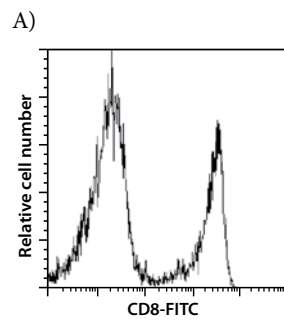
▲ Volumes given below are for **up to 10<sup>7</sup>** nucleated cells. When working with fewer than 10<sup>7</sup> cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2 $\times$ 10<sup>7</sup> nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Determine cell number.
2. Centrifuge cell suspension at 300 $\times$ g for 10 minutes. Aspirate supernatant completely.

3. Resuspend up to  $10^7$  nucleated cells per 100  $\mu\text{L}$  of buffer.
  - ▲ **Note:** (Optional) If CD8-PE or CD8-VioGreen is used, resuspend  $10^7$  nucleated cells in 80  $\mu\text{L}$  of buffer and add 20  $\mu\text{L}$  of FcR Blocking Reagent directly before addition of the CD8 antibody.
4. Add 10  $\mu\text{L}$  of the CD8 antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator ( $2-8^\circ\text{C}$ ).
  - ▲ **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  $300\times g$  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 CD8 antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with CD8 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. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



## 4. Reference

1. Jonker, M. and Slingerland, W. (1989) Reactivity of mAb specific for human CD markers with rhesus monkey leucocytes; in Knapp, W. *et al.* (eds.): Leukocyte Typing IV, Oxford, Oxford University Press.

All protocols and data sheets are available at [www.miltenyibiotec.com](http://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

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