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### 1. Description

<b>Components</b>	1 mL monoclonal CD8a antibodies, mouse conjugated to various dyes.
	FITC 130-091-605
	PE 130-091-603
	APC 130-091-606
	VioBlue® 130-094-360
	VioGreen™ 130-096-918
	PerCP 130-094-960
	PE-Vio770™ 130-097-025
	APC-Vio770 130-096-602
<b>Clone</b>	53-6.7 (isotype: rat 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.

#### 1.1 Background information

The mouse CD8a (Ly-2) antigen is expressed on cytotoxic T cells, and at lower levels on subpopulations of dendritic cells and TCR $\gamma/\delta^+$  cells. It is further detected on most thymocytes (CD4<sup>+</sup>CD8a<sup>+</sup> and CD4<sup>-</sup>CD8<sup>+</sup> thymocytes). Cytotoxic T cells and thymocytes express CD8a as heterodimer with CD8b, whereas dendritic cells and TCR $\gamma/\delta^+$  cells express a CD8a/CD8a homodimer. CD8a is expressed on approximately 10% of splenocytes, 25% of lymph node cells, and 85% of thymocytes in healthy mice.

### 1.2 Applications

- Identification and enumeration of CD8a<sup>+</sup> cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy, for example:
  - Positive selection and depletion of cytotoxic T cells by using CD8a MicroBeads (# 130-049-401), or the CD8a<sup>+</sup> T Cell Isolation Kit (# 130-090-859) for isolation of untouched cytotoxic T cells;
  - Isolation of mouse naive cytotoxic T cells by using CD8a<sup>+</sup> T Cell Isolation Kit (# 130-090-859) in combination with CD62L MicroBeads (# 130-049-701);
  - Isolation of mouse CD8a<sup>+</sup> dendritic cells by using the CD8<sup>+</sup> Dendritic Cell Isolation Kit (# 130-091-169).

### 1.3 Recommended antibody dilution

The recommended antibody dilution for all CD8a 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 CD8a MicroBead-labeled cells use the same dilution.

### 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 mouse serum albumin, mouse serum, or fetal bovine serum (FBS). Buffers or media containing Ca<sup>2+</sup> or Mg<sup>2+</sup> are not recommended for use.
- (Optional) FcR Blocking Reagent, mouse (# 130-092-575) 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.

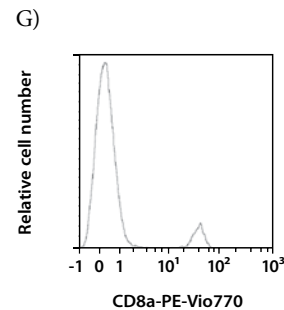
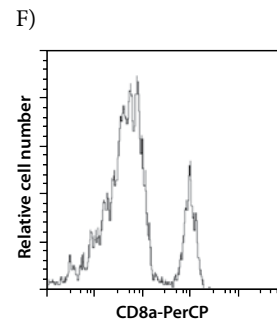
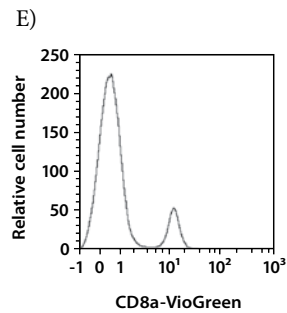
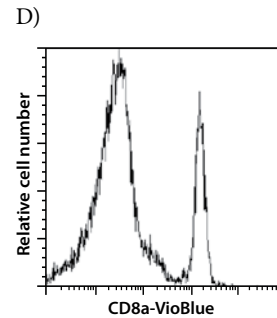
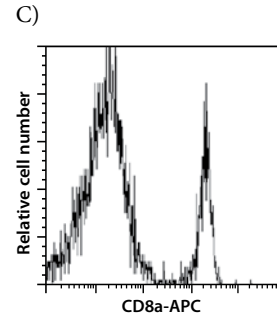
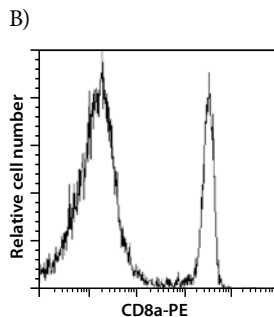
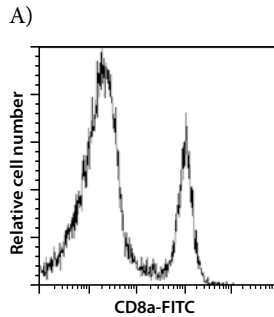
## 2. General protocol for immunofluorescent staining

▲ Volumes given below are for up to  $10^7$  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  $2 \times 10^7$  nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

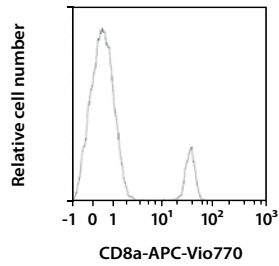
1. Determine cell number.
2. Centrifuge cell suspension at  $300 \times g$  for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to  $10^7$  nucleated cells per 100  $\mu\text{L}$  of buffer.
4. Add 10  $\mu\text{L}$  of the CD8a antibody.
5. Mix well and incubate for 10 minutes in the dark 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 CD8a antibodies

Mouse spleen cells were stained with CD8a 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<sup>®</sup> Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



H)



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.

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