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### 1. Description

<b>Components</b>	1 mL CD107a antibodies, human conjugated to various dyes.
	FITC 130-095-518
	PE 130-095-515
	APC 130-095-510
	VioBlue® 130-095-520
<b>Clone</b>	H4A3 (isotype: mouse IgG1k).
<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. Low endotoxin.
<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

CD107a, also known as lysosome-associated membrane protein 1 (LAMP-1), is a 110–140 kDa type I membrane glycoprotein. It is a widely expressed intracellular protein located in the lysosomal/endosomal membrane. CD107a transiently located on the cell membrane can be used as a marker of CD8<sup>+</sup> T cell degranulation following stimulation.<sup>1</sup> It is also expressed, to a lower extent, on activated NK cells.

#### 1.2 Applications

- Identification and enumeration of CD107a<sup>+</sup> cells by flow cytometry or fluorescence microscopy.
- Evaluation of intracellular cytokine expression of stimulated CD8<sup>+</sup> T cells by using CD107a antibodies in combination with antibodies against human cytokines .

#### 1.3 Recommended antibody dilution

The recommended antibody dilution for all CD107a conjugates is 1:11 for up to 10<sup>7</sup> cells/100 µL of buffer for labeling of cells and analysis by flow cytometry.

The antibody is suited for staining of formaldehyde-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 Ca<sup>2+</sup> or Mg<sup>2+</sup> are not recommended for use.
- Cell culture medium, for example, RPMI 1640 (# 130-091-440) containing 10% fetal bovine serum (FBS).
- Reagent for T cell stimulation, such as staphylococcal enterotoxin B (SEB).
- Secretion inhibitor, for example, monensin.
- Inside Stain Kit (# 130-090-477) for fixation and permeabilization of cells.
- (Optional) Fluorochrome-conjugated antibody for cell surface staining as well as intracellular staining, for example, CD8-APC (# 130-091-076) and Anti-IFN-γ-FITC (# 130-091-641). For more information about antibodies refer to [www.miltenyibiotec.com/antibodies](http://www.miltenyibiotec.com/antibodies).
- (Optional) Mouse IgG1-VioBlue (# 130-094-670), Mouse IgG1-FITC (# 130-092-213), Mouse IgG1-PE (# 130-092-212), or Mouse IgG1-APC (# 130-092-214) for isotype control.
- (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. Protocols

### 2.1 *In vitro* stimulation of CD107a-secreting cells

▲ Always include a negative control in the experiment. The sample should be treated in exactly the same manner as the stimulated sample, except for the addition of the stimulus.

1. Wash cells by adding medium and centrifuge at 300×g for 10 minutes. Aspirate supernatant.
2. Resuspend cells at a density of 2×10<sup>7</sup> per mL in culture medium containing 5% human serum. Plate 50 μL of cells in a 96-well dish.
3. Add antigen or control reagent in the appropriate concentration, for example, 1 μg/mL SEB.
4. Add monensin for secretion inhibition (titer: 1:1000).
5. Add 10 μL of CD107a antibodies.
6. Add medium to a total volume of 100 μL (cell density of 1×10<sup>6</sup> total cells/mL).
4. Incubate cells for 5 hours at 37 °C and 5% CO<sub>2</sub>.
5. Collect cells carefully by pipetting up and down. Rinse the dish with cold buffer. Check microscopically for any remaining cells, if necessary, rinse the dish again.
6. Proceed to immunofluorescent staining (2.2).

### 2.2 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 2×10<sup>7</sup> nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

#### 2.2.1 Cell surface staining

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10<sup>7</sup> nucleated cells per 100 μL of buffer.
4. Add staining antibodies according to the manufacturer's recommendations, for example, CD8-APC (# 130-091-076).
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.

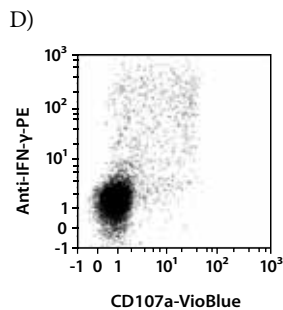
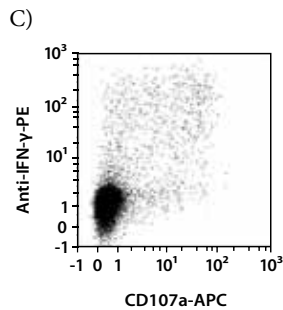
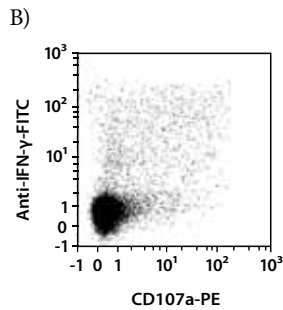
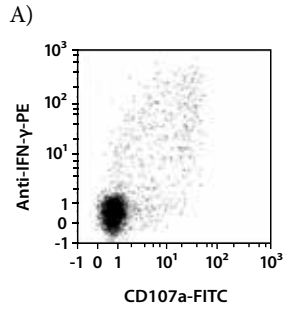
#### 2.2.2 Intracellular staining of cells in suspension

▲ It is recommended to stain 10<sup>6</sup> cells per sample. When working with up to 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 2×10<sup>7</sup> nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Wash up to 10<sup>7</sup> cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
2. (Optional) Stain cell surface antigens that are sensitive to fixation with appropriate antibodies according to the manufacturer's recommendations. Then wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10<sup>7</sup> cells in 500 μL of buffer.
4. Add 500 μL of Inside Fix (Inside Stain Kit). Mix well and incubate for 20 minutes in the dark at room temperature.
5. Wash cells by adding 1 mL of Inside Perm (Inside Stain Kit) and centrifuge at 300×g for 5 minutes. Aspirate supernatant carefully.  
▲ **Note:** Fixed cells may be stored in azide-containing buffer at 2–8 °C for up to 1 week.
6. Resuspend cells in 100 μL of Inside Perm.
7. (Optional) Add additional staining antibodies to the solution, for example, 10 μL of CD8-APC (# 130-091-076) and 10 μL of Anti-IFN-γ-FITC (# 130-091-641).  
▲ **Note:** For efficient permeabilization upon intracellular staining the volume of Inside Perm should be at least 5× the volume of staining antibodies.
8. Mix well and incubate for 10 minutes in the dark at room temperature.
9. Wash cells by adding 1 mL of Inside Perm and centrifuge at 300×g for 5 minutes. Aspirate supernatant carefully.
10. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy. Store cells at 2–8 °C in the dark until analysis. Mix well before flow cytometric acquisition.  
▲ **Note:** Samples may be stored at 2–8 °C in the dark for up to 24 hours.  
▲ **Note:** Do not use propidium iodide (PI) or 7-AAD staining.

### 3. Examples of immunofluorescent staining with CD107a antibodies

Human PBMCs were stimulated *in vitro* for 5 hours with SEB in the presence of monensin and CD107a antibodies conjugated to FITC (A), PE (B), APC (C), or VioBlue (D). Cells were harvested, fixed, permeabilized, and intracellularly stained with Anti-IFN- $\gamma$  antibodies. Additionally, cell surface staining was performed with CD8 antibodies. Cells were analyzed by flow cytometry using the MACSQuant<sup>®</sup> Analyzer. CD8<sup>+</sup>, IFN- $\gamma$ -secreting cells are shown.



### 4. Reference

1. Betts, M.R. *et al.* (2003) Sensitive and viable identification of antigen-specific CD8<sup>+</sup> T cells by a flow cytometric assay for degranulation. *J. Immunol. Methods* 281: 65–78.

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

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