Miltenyi Biotec

CD107a (LAMP-1) antibodies
human

Contents

1. Description

This product is for research use only.

Components Monoclonal CD107a (LAMP-1) antibodies, human conjugated to:

Conjugate	Order no. 1 mL (100 tests)	Order no. 300 µL (30 tests)
FITC	130-095-518	130-099-394
VioBright™ FITC	130-106-233	130-106-265
PE	130-095-515	130-099-415
APC	130-095-510	130-100-314
VioBlue®	130-095-520	130-100-351
APC-Vio® 770	130-106-102	130-106-153
Vio 515	130-109-114	130-109-160
Biotin	130-106-237	130-106-269

Clone H4A3 (isotype: mouse IgG1k).

Capacity 1 mL: 100 tests or up to 10^9 total cells

300 µL: 30 tests or up to 3×10^8 total cells.

Product format Antibodies are supplied in buffer containing stabilizer. Low endotoxin. Azide-free. Always handle sterile.

Storage Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

Cross-reactivity: The CD107a (LAMP-1) antibody has been reported to react with

- rhesus monkey (*Macaca mulatta*) cells
- pigtail monkey (*Macaca nemestrina*) cells
- african green monkey (*Chlorocebus aethiops*) cells
- chimpanzee (*Pan troglodytes*) cells
- baboon cells

1.1 Background information

- Antigen: CD107a (LAMP-1)
- Synonym: LAMP-1, LAMPA, LGP120
- Expression patterns: Clone H4A3 recognizes the CD107a antigen, also known as lysosome-associated membrane protein 1 (LAMP-1), 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 plasma membrane can be used as a marker for $CD8^+$ T cell degranulation following stimulation.[1] It is also expressed to a lower extent on activated NK cells.

1.2 Applications

- Identification and enumeration of CD107a $(LAMP\text{-}1)^+$ cells by flow cytometry or fluorescence microscopy.
- Evaluation of intracellular cytokine expression of stimulated $CD8^+$ T cells by using CD107a (LAMP-1) antibodies in combination with antibodies against human cytokines.

1.3 Recommended antibody dilution

The recommended antibody dilution for all CD107a (LAMP-1) conjugates is **1:11 for up to 10^7 cells/100 µL** of buffer for labeling of cells and subsequent 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^{2+} or Mg^{2+} are not recommended for use.

- (Optional) Tandem Signal Enhancer, human (# 130-099-888) to reduce non-specific binding of tandem dye–conjugated antibodies to human cells, especially to monocytes.

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- Cell culture medium, for example, RPMI 1640 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 and Anti-IFN-γ-FITC. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Mouse IgG1 antibodies conjugated to, e.g., VioBlue® for isotype control.
- (Optional) Conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE as secondary antibody reagent in combination with CD107a (LAMP-1)-Biotin.
- (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 (LAMP-1)–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 2×10^7 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 (LAMP-1) antibodies.
6. Add medium to a total volume of 100 µL (cell density of 1×10^6 total cells/mL).
4. Incubate cells for 5 hours at 37 °C and 5% CO_2.
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 10^7 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).

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 10^7 nucleated cells per 100 µL of buffer.
4. Add staining antibodies according to the manufacturer's recommendations, for example, CD8-APC.
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 10^6 cells per sample. When working with up to 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. Wash up to 10^7 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 10^7 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 and 10 µL of Anti-IFN-γ-FITC.

   ▲ **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.

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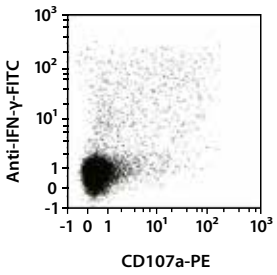
9. Wash cells by adding 1 mL of Inside Perm and centrifuge at 300×g for 5 minutes. Aspirate supernatant carefully.
10. (Optional) If CD107a (LAMP-1)-Biotin was used, resuspend cell pellet in 100 µL of Inside Perm, add 10 µL of anti-biotin antibody, and continue as described in steps 8 and 9.
11. Resuspend 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 (LAMP-1) antibodies

Human peripheral blood mononuclear cells (PBMCs) were stimulated *in vitro* for 5 hours with SEB in the presence of monensin and CD107a (LAMP-1) antibodies. Cells were harvested, fixed, permeabilized, and intracellularly stained with Anti-IFN-γ antibodies. Additionally, cell surface staining was performed with CD8 antibodies. Cells were analyzed by flow cytometry using the MACSQuant® Analyzer. $CD8^+$, IFN-γ–secreting cells are shown.



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

4. Reference

1. Betts, M.R. *et al.* (2003) Sensitive and viable identification of antigen-specific $CD8^+$ 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**.

Warranty