



CD39 antibodies human

CD39-FITC	130-093-502
CD39-PE	130-093-503
CD39-APC	130-093-504
CD39-Biotin	130-093-505
CD39 pure	130-093-506

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1. Description

Components	1 mL CD39 antibodies, human: monoclonal CD39 antibodies conjugated to fluorescein isothiocyanate (FITC), R-phycoerythrin (PE), allophycocyanin (APC), or biotin. The unconjugated (pure) antibody is supplied at a concentration of 100 µg/mL.
Clone	MZ18-23C8 (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.

1.1 Background information

The CD39 antibody specifically recognizes the membrane-bound human CD39 antigen, which is expressed on an effector/memory-like subset of FoxP3⁺ regulatory T cells¹, monocytes, and B cell subsets. CD39 is an ectonucleotidase and catalyzes the hydrolysis of extracellular nucleotides, for example, ATP. In concert with CD73, which is an ecto-5'-nucleotidase, this can lead to the production of adenosine.

High extracellular ATP concentrations indicate tissue injury and cell death and induce various pro-inflammatory responses in immune cells.

Through its enzymatic activity, CD39 can contribute to the suppressive function of regulatory T cells, for example, by eliminating extracellular ATP or by generating adenosine, which has suppressive effects on various immune cells.

1.2 Applications

- Identification and enumeration of CD39⁺ cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy. For the evaluation of CD39 expression on regulatory T cells, CD4⁺CD25⁺ regulatory

T cells can be enriched by using, for example, the CD4⁺CD25⁺ Regulatory T Cell Isolation Kit, human (# 130-091-301) and stained with CD39 antibodies.

1.3 Recommended antibody dilution

For antibody labeling of human cells.

CD39 conjugate	PE	APC	Biotin
Flow cytometry^a			
- In general	1:11	1:11	1:11
- Formaldehyde-fixed cells	1:11	1:11	1:11

a) Given antibody dilutions are for a cell concentration of up to 10⁷ cells/100 µL of buffer.

- Cross-reactivity: The CD39 antibody is tested to react with rhesus monkey (*Macaca mulatta*).

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. 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) Anti-Biotin-FITC (# 130-090-857), Anti-Biotin-PE (# 130-090-756), or Anti-Biotin-APC (# 130-090-856) as secondary antibody reagent in combination with CD39-Biotin.
- (Optional) CD4-FITC (# 130-080-501), CD4-PE (# 130-091-231), CD25-APC (# 130-092-858), Anti-FoxP3-PE (# 130-093-014), or Anti-FoxP3-APC (# 130-093-013).
- (Optional) Propidium iodide (PI) 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.

140-003-215-03



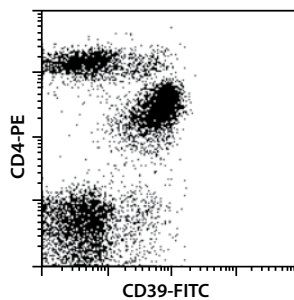
3. Resuspend up to 10^7 nucleated cells per 100 μL of buffer.
4. Add 10 μL of the CD39 antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 $^{\circ}\text{C}$).

▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.
6. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at $300\times g$ for 10 minutes. Aspirate supernatant completely.
7. (Optional) If CD39-Biotin was used, resuspend the cell pellet in 100 μL of buffer, add 10 μL of anti-biotin antibody (Anti-Biotin-FITC, Anti-Biotin-PE, or Anti-Biotin-APC), and continue as described in steps 5 and 6.
8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

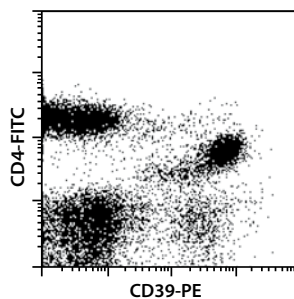
3. Examples of immunofluorescent staining with CD39 antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with CD39 antibodies conjugated to FITC (a), PE (b), or APC (c), as well as with CD4-PE (# 130-091-231) and CD4-FITC (# 130-080-501) and analyzed by flow cytometry. Cells stained with CD39-Biotin (d) were stained with Anti-Biotin-APC (# 130-090-856) as well as CD4-FITC. Additionally, a staining with CD39-APC and Anti-FoxP3-PE is shown, gated on CD4⁺ T cells (e). Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

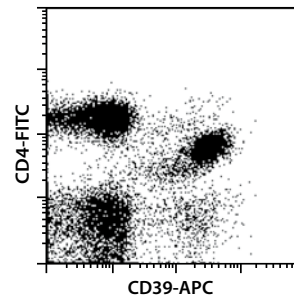
(a) Human PBMCs stained with CD39-FITC and CD4-PE.



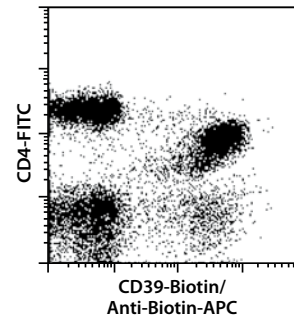
(b) Human PBMCs stained with CD39-PE and CD4-FITC.



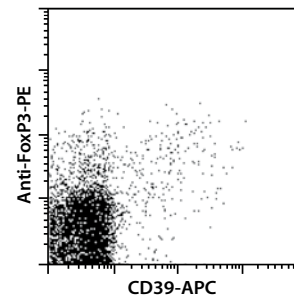
(c) Human PBMCs stained with CD39-APC and CD4-FITC.



(d) Human PBMCs stained with CD39-Biotin, Anti-Biotin-APC, and CD4-FITC.



(e) Human PBMCs stained with CD39-APC, CD4-FITC, and Anti-FoxP3-PE.



4. Reference

1. Borsellino *et al.* (2007) Expression of ectonucleotidase CD39 by Foxp3⁺ Treg cells: hydrolysis of extracellular ATP and immune suppression. *Blood* 110: 1225–1232. [10229]

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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