

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 CD123 antibodies

1. Description

Components	1 mL monoclonal CD123 antibodies, human conjugated to various dyes.
	FITC 130-090-897
	PE 130-090-899
	APC 130-090-901
	VioBlue® 130-097-330
	pure 130-090-940
Clone	AC145 (isotype: mouse IgG2a).
Capacity	100 tests or up to 10 ⁹ total cells. The unconjugated (pure) antibody is supplied at a concentration of 50 µg/mL.
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 CD123 antigen, which is also known as IL-3 receptor alpha chain, interacts with the common beta chain (CD131) of the IL-3, IL-5, and GM-CSF receptors to form a high affinity IL-3 receptor. CD123 alone binds IL-3 with low affinity. Dependent on the cell type, binding of IL-3 to the high affinity IL-3 receptor heterodimer stimulates cell proliferation, cell differentiation, or cell survival.

In peripheral blood, CD123 is expressed at high levels only on plasmacytoid dendritic cells and basophils, but at lower levels also on monocytes, eosinophils, myeloid dendritic cells, and subsets of hematopoietic progenitor cells (multipotent and myeloid precursors, but not lymphoid precursors).

1.2 Applications

- Identification and enumeration of CD123⁺ cells by flow cytometry or fluorescence microscopy.
- Flow cytometric and fluorescence microscopic identification and enumeration of basophil purity after the isolation of basophils using the Basophil Isolation Kit II (# 130-092-662).

Stain purified basophils with a CD123 antibody and an antibody against BDCA-2 coupled to another fluorochrome. In this case, basophils represent a discrete population, which is CD123^{bright}BDCA-2⁻.

1.3 Recommended antibody dilution

The recommended antibody dilution for all C123y conjugates is **1:11 for up to 10⁷ cells/100 µL** of buffer for labeling of cells and analysis by flow cytometry. For CD123 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 Ca²⁺ or Mg²⁺ are not recommended for use.
- FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor-mediated antibody labeling.
- (Optional) CD303 (BDCA-2)-FITC (# 130-090-510) or CD303 (BDCA-2)-PE (# 130-090-511). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (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

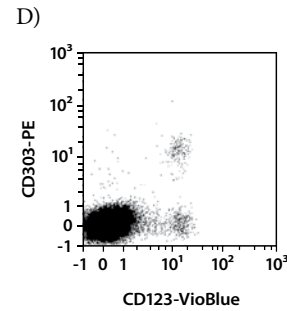
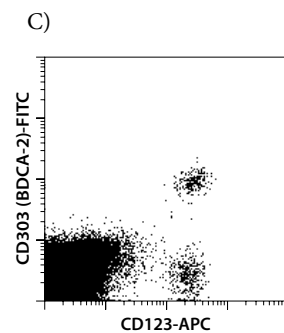
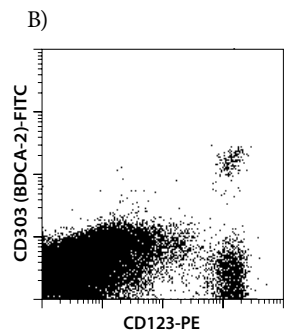
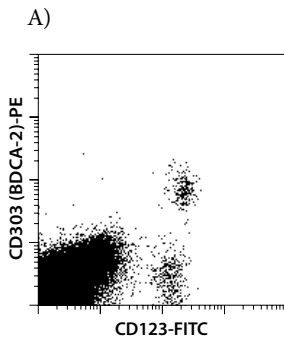
▲ Volumes given below are for up to 10⁷ nucleated cells. When working with fewer than 10⁷ cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10⁷ nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10⁷ nucleated cells per 80 µL of buffer.
4. Add 20 µL FcR Blocking Reagent.

5. Add 10 μ L of the CD123 antibody.
6. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 $^{\circ}$ C).
 - ▲ Note:** Higher temperatures and/or longer incubation times lead to non-specific cell labeling. Working on ice requires increased incubation times.
7. 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.
8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

3. Examples of immunofluorescent staining with CD123 antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with CD123 antibodies conjugated to FITC (A), PE (B), APC (C), or VioBlue (D) as well as with CD303 (BDCA-2)-FITC (# 130-090-510) or CD303 (BDCA-2)-PE (# 130-090-511) and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



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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