



Antibodies

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1. Description

Clone A3C6E2 (isotype: mouse IgG1).

Product format 1 mL CD117 antibodies, human: monoclonal CD117 antibodies conjugated to R-phycoerythrin (PE), or allophycocyanin (APC). The antibodies are supplied in a solution containing stabilizer and 0.05% sodium azide.

Product size 100 tests (for up to 10^9 nucleated cells).

Storage Store protected from light at 4–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Background and product applications

The CD117-specific monoclonal antibody (mAb) clone A3C6E2 recognizes the human CD117 antigen. It recognizes a CD117 epitope which is different from the epitope recognized by the CD117 mAb clone AC126. Therefore, mAb clone A3C6E2 is recommended for fluorescent-control staining for cells labeled with of the CD117 MicroBead Kit, human (# 130-091-332). CD117 (also known as c-kit, steel factor receptor or SCF receptor) is a 145 kDa cell surface glycoprotein with tyrosine kinase activity. This molecule is suggested to be involved in signaling, activation, and proliferation of cells. The CD117 antigen is expressed on about 1–3% of peripheral blood mononuclear cells (PBMCs) and cord blood cells and up to 10% of bone marrow cells. Around 25% of CD117⁺ cells were found to express CD133 and CD34. CD117 is further expressed on basophils, myeloid dendritic cells, TCRαβ⁺ T cells, CD19⁺ B cells, and CD56⁺ NK cells¹ as well as on mast cells, melanocytes, and AML (acute myeloid leukemia) blasts². The CD117-specific monoclonal antibody A3C6E2 potentially interferes with stem cell factor (SCF)-binding. Therefore, it is recommended to stain aliquots of the cell sample and to use unstained cells for further experiments. Fluorescent staining of CD117⁺ cells, intended to be used for further experiments, requires antibodies that do not interfere with stem cell factor (SCF)-binding. For this application, the use of CD117 (AC126)-PE (# 130-091-735) is recommended.

CD117 antibodies

human

CD117(A3C6E2)-PE
CD117(A3C6E2)-APC

130-091-734
130-091-733

Product applications

- Identification and enumeration of CD117 cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® separations by flow cytometry or fluorescence microscopy, for example positive selection or depletion of human stem cells by using the CD117 MicroBead Kit, human (# 130-091-332).

1.2 Examples of staining concentrations for human cells.

CD117 (A3C6E2) conjugate	PE	APC
	Recommended antibody dilution	
Flow cytometry^a		
- in general	1:11	1:11
- formaldehyde-fixed cells ^b	1:11	1:11
- CD117 MicroBead-labeled cells	1:11	1:11
<small>a) Given antibody dilutions are for a cell concentration of up to 1×10^8 cells/mL buffer. b) For optimal results, human cells have to be stained prior to fixation.</small>		

1.3 Reagent requirements

- Buffer: Prepare a solution containing PBS (phosphate buffered saline) pH 7.2, 0.5% BSA and 2 mM EDTA, e.g. by diluting MACS BSA Stock Solution (#130-091-376) 1:20 with autoMACS™ Rinsing Solution (# 130-091-222). Keep buffer cold (4–8 °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 calf serum. Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- FcR Blocking Reagent, human (# 130-059-901): Fc receptor-mediated fluorescent staining can be avoided by blocking of Fc receptor using FcR Blocking Reagent, human.
- (Optional) PI (propidium iodide) or 7-AAD for flow cytometric exclusion of dead cells without cell fixation. For cell fixation and flow cytometric exclusion of dead cells, the Fixation and Dead Cell Discrimination Kit (# 130-091-163) is recommended.

2. General protocol for immunofluorescent staining

▲ Volumes for fluorescent labeling given below are for up to 10^7 total 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 total cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Resuspend up to 10^7 nucleated cells per 80 µL of buffer.
2. Add 20 µL of FcR Blocking Reagent.

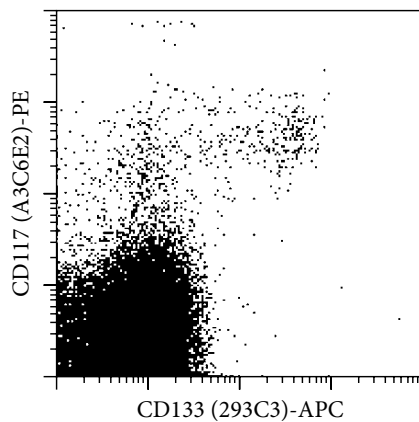


3. Add 10 μ L of CD117 antibodies.
4. Mix well and incubate for 10 minutes in the dark at 4–8 °C.
 ▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.
5. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300 \times g for 10 minutes. Pipette off supernatant completely.
6. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

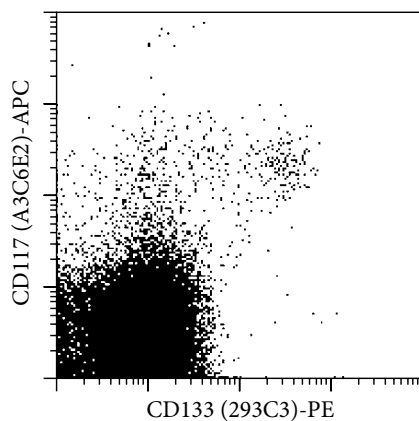
3. Examples of immunofluorescent staining with CD117 antibodies

Human cord blood mononuclear cells (CB MNCs) were stained with CD117 antibodies conjugated to PE (a) or APC (b) and CD133 antibodies conjugated to APC (a) or PE (b), and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

(a) Human CB MNCs stained with CD117 (A3C6E2)-PE.



(b) Human CB MNCs stained with CD117 (A3C6E2)-APC.



4. References

1. Guth S., Miltenyi S., Schmitz, J. (1995) Immunomagnetic isolation and surface phenotyping of human c-kit receptor-expressing cells from peripheral blood. (Abstract) 9th International Congress of Immunology
2. Bühring H. J., Ullrich A., Schaudt K., Müller C. A., Busch F. W. (1991) The product of the proto-oncogene c-kit (P145c-kit) is a human bone marrow surface antigen of hematopoietic precursor cells which is expressed on a subset of acute non-lymphoblastic leukemic cells. *Leukemia* 5: 854.

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.

Warranty

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