



## Antibodies

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### 1. Description

<b>Clone</b>	AC126 (isotype: mouse IgG1).
<b>Product format</b>	1 mL CD117 antibodies, human: monoclonal CD117 antibodies conjugated to R-phycoerythrin (PE). The antibodies are supplied in a solution containing stabilizer and 0.05% sodium azide.
<b>Product size</b>	100 tests (for up to $10^9$ nucleated cells).
<b>Storage</b>	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) AC126 recognizes the human CD117 antigen. It recognizes a CD117 epitope which is identical or in close proximity to the epitope recognized by the CD117 mAb clone 104D2. This is different from the epitope recognized by the CD117 mAb clone A3C6E2. 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<sup>+</sup> cells were found to express CD133 and CD34. CD117 is further expressed on basophils, myeloid dendritic cells, TCRαβ<sup>+</sup> T cells, CD19<sup>+</sup> B cells, and CD56<sup>+</sup> NK cells<sup>1</sup> as well as on mast cells, melanocytes, and AML (acute myeloid leukemia) blasts<sup>2</sup>. mAb AC126 does not interfere with stem cell factor (SCF)-binding. Fluorescent control staining of human cells labeled by using the CD117 MicroBead Kit, human (# 130-091-332) requires a mAb recognizing a CD117 epitope, which is different from the epitope recognized by the mAb clone AC126. For this, the fluorochromes CD117 (A3C6E2)-PE, human (# 130-091-734) and CD117 (A3C6E2)-APC, human (# 130-091-733) are recommended.

#### Product applications

- Identification and enumeration of CD117 cells by flow cytometry or fluorescence microscopy.
- Indirect isolation of CD117<sup>+</sup> cells by using CD117 (AC126)-PE (# 130-091-735) and Anti-PE MicroBeads (# 130-048-801).

# CD117 (AC126)-PE human

CD117 (AC126)-PE

130-091-735

### 1.2 Examples of staining concentrations for human cells.

CD117 (AC126) conjugate	PE
Recommended antibody dilution	
Flow cytometry <sup>a</sup>	
- in general	1:11
- formaldehyde-fixed cells <sup>b</sup>	1:11
- CD117 MicroBead-labeled cells	n.r.

a) Given antibody dilutions are for a cell concentration of up to  $1 \times 10^8$  cells/mL buffer.  
b) For optimal results, human cells have to be stained prior to fixation.  
n.r. Not recommended.

### 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 Ca<sup>2+</sup> or Mg<sup>2+</sup> 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  $2 \times 10^7$  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 µL of CD117 antibodies.  
▲ **Note:** See table for exceptions.
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×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.

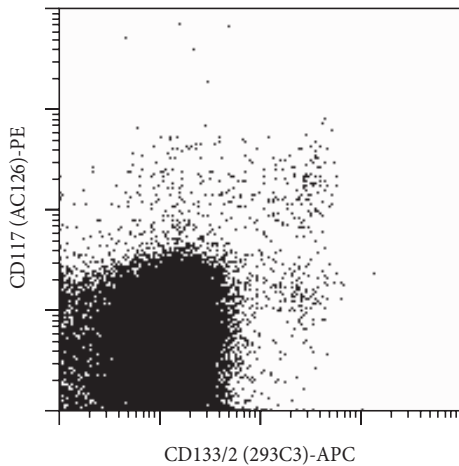
140-001-251.01



### 3. Examples of immunofluorescent staining with CD117 antibodies

Human cord blood mononuclear cells (CB MNCs) were stained with CD117 antibodies (clone AC126) conjugated to PE and CD133 antibodies (293C3) conjugated to APC, and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

Human CB MNCs stained with CD117 (AC126)-PE



### 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 HJ, Ullrich A, Schaudt K, Müller CA, Busch FW (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.

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