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## 1. Description

<b>Components</b>	1 mL CD117 antibodies, mouse: monoclonal CD117 antibodies conjugated to R-phycoerythrin (PE), or allophycocyanin (APC).
<b>Clone</b>	3C1 (isotype: rat IgG2b).
<b>Capacity</b>	100 tests or up to 10 <sup>9</sup> total cells.
<b>Product format</b>	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.
<b>Storage</b>	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

### 1.1 Background information

CD117, also known as c-kit, steel factor receptor, and stem cell factor receptor, encodes a 145 kDa cell surface glycoprotein belonging to the class III receptor tyrosine kinase family. It is expressed on the majority of hematopoietic progenitor cells, including multipotent hematopoietic stem cells as well as committed myeloid, erythroid, and lymphoid precursor cells. In addition to the hematopoietic cell differentiation potential, CD117<sup>+</sup> stem cells from mouse bone marrow were reported to be capable of differentiation into smooth muscle cells, myocytes, and endothelial cells *in vivo*.<sup>1,2</sup> CD117 is also expressed on a few mature hematopoietic cells, for example, mast cells.

### 1.2 Applications

- Identification and enumeration of CD117<sup>+</sup> cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy, for example:
  - Positive selection or depletion of mouse stem cells by using CD117 MicroBeads, mouse (# 130-091-224) or the Anti-Sca-1 MicroBead Kit (FITC), mouse (# 130-092-529).
  - Isolation of mouse lineage marker negative cells by using the Lineage Cell Depletion Kit, mouse (# 130-090-858).

### 1.3 Recommended antibody dilution

For antibody labeling of mouse cells.

CD117 conjugate	PE	APC
<b>Flow cytometry<sup>a</sup></b>		
- In general	1:11	1:11
- Formaldehyde-fixed cells <sup>b</sup>	1:11	1:11
- CD117 MicroBead-labeled cells	1:11	1:11
<b>Immunohistochemistry<sup>c</sup></b>		
a) Given antibody dilutions are for a cell concentration of up to 10 <sup>7</sup> cells/100 µL of buffer.		
b) For optimal results, cells must be stained prior to fixation.		
c) The optimal antibody dilution should be determined.		

### 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 mouse serum albumin, mouse serum, or fetal bovine serum. Buffers or media containing Ca<sup>2+</sup> or Mg<sup>2+</sup> are not recommended for use.
- (Optional) FcR Blocking Reagent, mouse (# 130-092-575) to avoid Fc receptor-mediated antibody labeling.
- (Optional) Anti-Sca-1-FITC (# 130-093-222), Anti-Sca-1-PE (# 130-093-224), Anti-Sca-1-APC (# 130-093-223), or Anti-Sca-1-Biotin (# 130-093-421).
- (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 10<sup>7</sup> nucleated cells. When working with fewer than 10<sup>7</sup> 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<sup>7</sup> 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 10<sup>7</sup> nucleated cells per 100 µL of buffer.
4. Add 10 µL of the CD117 antibody.

5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).

▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

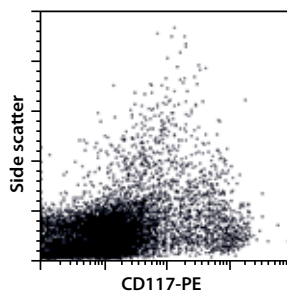
6. Wash cells by adding 1–2 mL of buffer per  $10^7$  cells and centrifuge at  $300\times g$  for 10 minutes. Aspirate supernatant completely.

7. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

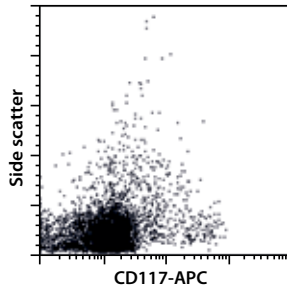
### 3. Examples of immunofluorescent staining with CD117 antibodies

Mouse bone marrow cells were stained with CD117 antibodies conjugated to PE (a), or APC (b), and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

(a) Mouse bone marrow cells stained with CD117-PE.



(b) Mouse bone marrow cells stained with CD117-APC.



### 4. References

1. Orlic, D. (2002) Stem cell repair in ischemic heart disease: an experimental model. *Int. J. Hematol.* 76, Suppl. 1: 144–145.
2. Orlic D. *et al.* (2001) Mobilized bone marrow cells repair the infarcted heart, improving function and survival. *Proc. Natl. Acad. Sci. U S A* 98: 10344–10349.

All protocols and data sheets are available at [www.miltenyibiotec.com](http://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.

#### Warranty

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