

# CD117 antibodies, mouse

### For research use only

9 μg equal 60 tests, 30 μg equal 200 tests. One test corresponds to labeling of 10<sup>6</sup> cells.

Product	Content	Order no.
CD117-PE	9 μg in 300 μL	130-102-795
CD117-PE	30 μg in 1 mL	130-102-542
CD117-APC	9 μg in 300 μL	130-102-796
CD117-APC	30 μg in 1 mL	130-102-492
CD117-PE-Vio770	9 μg in 300 μL	130-108-384
CD117-PE-Vio770	30 μg in 1 mL	130-108-355
CD117-Biotin	9 μg in 300 μL	130-102-000
CD117-Biotin	30 μg in 1 mL	130-101-930

#### 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.

## Technical data and background information

 Antigen
 CD117

 Clone
 3C11

 Isotype
 rat IgG2b

**Isotype control** Rat IgG2b – isotype control antibodies

Alternative names of antigen c-kit, SCO1, SCO5, SOW3, Ssm, Tr-kit, W, KIT, SCFR

Molecular mass of antigen [kDa] 107

**Distribution of antigen**B cells, bone marrow, brain, cancer stem cells, dendritic cells,

endothelial cells, fibroblasts, hematopoietic stem cells, kidney, leukemia cells, liver, lung, mast cells, megakaryocytes, myeloid

cells, NK cells, red blood cells, T cells

Product format Antibodies are supplied in buffer containing stabilizer and 0.05%

sodium azide.

**Fixation** Cells should be stained prior to fixation, if formaldehyde is used

as a fixative.

**Storage** Store protected from light at 2–8 °C. Do not freeze.

Clone 3C1 recognizes mouse CD117, a 145 kDa cell surface glycoprotein belonging to the class III receptor tyrosine kinase family. CD117 is also known as c-kit and stem cell factor (SCF) receptor. It is expressed on the majority of hematopoietic progenitor cells including multipotent hematopoietic stem cells as well as some committed myeloid, erythroid and lymphoid precursor cells, and mature mast

cells. CD117<sup>+</sup> stem cells from murine bone marrow could also be differentiated into smooth muscle cells, myocytes, and endothelial cells *in vivo*.<sup>1,2</sup>

## Reagent requirements

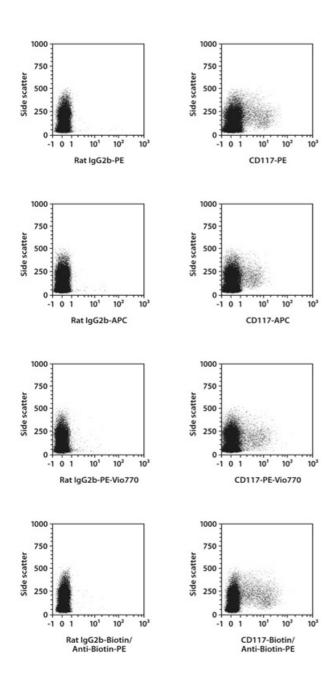
- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS<sup>®</sup> BSA Stock Solution (# 130-091-376) 1:20 with autoMACS<sup>®</sup> Rinsing Solution (# 130-091-222). Keep buffer cold (2-8 °C). Note: EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). 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) Fluorochrome-conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with biotinylated antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) 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.

#### Protocol for cell surface staining

- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:10 for up to 10<sup>6</sup> cells/50 µL of buffer.
- Volumes given below are for up to 10<sup>6</sup> nucleated cells. When working with fewer than 10<sup>6</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>6</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  $10^6$  nucleated cells per 45  $\mu$ L of buffer.
- 4. Add 5 μL of the antibody.
- 5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2-8 °C). Note: Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.
- 6. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
- 7. (Optional) If biotinylated antibody was used, resuspend the cell pellet in 100  $\mu$ L of buffer, add 10  $\mu$ L of fluorochrome-conjugated anti-biotin antibody, 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.

### **Examples of immunofluorescent staining**

Mouse bone marrow suspension cells were stained with CD117 antibodies or with the corresponding isotype control antibodies (left image). Flow cytometry was performed with the MACSQuant<sup>®</sup> Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence or 4',6-diamidino-2-phenylindole (DAPI) fluorescence, as in the case of tandem conjugates.



#### References

- 1. Orlic, D. (2002) Stem cell repair in ischemic heart disease: an experimental model. Int. J. Hematol. 76(suppl.1): 144–145.
- 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.

#### Warranty

The products sold hereunder are warranted only to be free from defects in workmanship and material at the time of delivery to the customer. Miltenyi Biotec GmbH makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the technical specifications of the products. Miltenyi Biotec GmbH's liability is limited to either replacement of the products or refund of the purchase price. Miltenyi Biotec GmbH is not liable for any property damage, personal injury or economic loss caused by the product.

Miltenyi Biotec GmbH | Friedrich-Ebert-Straße 68 | 51429 Bergisch Gladbach | Germany | Phone +49 2204 8306-0 | Fax +49 2204 85197 | macs@miltenyibiotec.de | www.miltenyibiotec.com

Miltenyi Biotec provides products and services worldwide. Visit www.miltenyibiotec.com/local to find your nearest Miltenyi Biotec contact.

Unless otherwise specifically indicated, Miltenyi Biotec products and services are for research use only and not for therapeutic or diagnostic use. autoMACS, MACS, MACSQuant, Vio, VioBlue, VioBright, and VioGreen are either trademarks or registered trademarks of Miltenyi Biotec GmbH. Copyright © 2016 Miltenyi Biotec GmbH. All rights reserved.