



# Anti-MSCA-1 (W8B2) antibodies human

Anti-MSCA-1 (W8B2)-PE	130-093-587
Anti-MSCA-1 (W8B2)-APC	130-093-589
Anti-MSCA-1 (W8B2)-Biotin	130-093-593
Anti-MSCA-1 (W8B2) pure	130-093-595

## 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 Anti-MSCA-1 (W8B2) antibodies
4. References

Tool Box – MSCA-1 (W8B2) (# 130-093-572) including reagents for the isolation and optimized expansion of MSCA-1 (W8B2)<sup>+</sup> MSCs.

### 1.3 Recommended antibody dilution

For antibody labeling of human cells.

Anti-MSCA-1 (W8B2) conjugate	PE	APC	Biotin
<b>Flow cytometry<sup>a</sup></b>			
- In general	1:11	1:11	1:11
- Formaldehyde-fixed cells <sup>b</sup>	1:11	1:11	1:11
- Anti-MSCA-1 (W8B2) MicroBead-labeled cells	1:11	1:11	1:11

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.

## 1. Description

<b>Components</b>	1 mL Anti-MSCA-1 (W8B2) antibodies, human: monoclonal Anti-MSCA-1 (W8B2) antibodies conjugated to R-phycoerythrin (PE), allophycocyanin (APC), or biotin. The unconjugated (pure) antibody is supplied at a concentration of 100 µg/mL.
<b>Clone</b>	W8B2 (isotype: mouse IgG1).
<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

Monoclonal antibody clone W8B2 recognizes the mesenchymal stromal cell antigen 1 (MSCA-1), a so far unknown antigen. MSCA-1 was shown to be restricted to mesenchymal stromal cells (MSCs) in the CD271<sup>bright</sup> population in bone marrow.<sup>1</sup> These CD271<sup>bright</sup>CD45<sup>dim</sup> MSCs have a much higher clonogenic capacity compared to the CD271<sup>+</sup>CD45<sup>+</sup> fraction in bone marrow.<sup>1</sup> Therefore, MSCA-1 is a suitable marker to identify MSCs with a high proliferative potential. MSCA-1 expression was not found on placenta-derived MSCs after culture.<sup>2</sup>

### 1.2 Applications

- Identification and enumeration of MSCs with high proliferative capacity from bone marrow or other tissue such as lipoaspirate by flow cytometry or fluorescence microscopy. The antibody is suitable for MACS<sup>®</sup> Control applications.
- Evaluation of MACS Separations by flow cytometry or fluorescence microscopy. Human MSCA-1 (W8B2)<sup>+</sup> cells can be isolated by using, for example, the Anti-MSCA-1 (W8B2) MicroBead Kit, human (# 130-093-583), or the MSC Research

### 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<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 phosphate 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. Buffers or media containing Ca<sup>2+</sup> or Mg<sup>2+</sup> are not recommended for use.
- (Optional) FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor-mediated antibody labeling.
- (Optional) Anti-Biotin-FITC (# 130-090-857), Anti-Biotin-PE (# 130-090-756), or Anti-Biotin-APC (# 130-090-856) as secondary antibody reagent in combination with Anti-MSCA-1 (W8B2)-Biotin.
- (Optional) CD271 (LNGFR)-PE (# 130-091-885), CD271 (LNGFR)-APC (# 130-091-884), CD45-FITC (# 130-080-202), CD45-PE (# 130-080-201), CD45-APC (# 130-091-230), or CD45-VioBlue (# 130-092-880).
- (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).

140-002-234/01



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. (Optional) Add 20 μL of FcR Blockig Reagent.
5. Add 10 μL of the Anti-MSCA-1 (W8B2) antibody.
6. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).

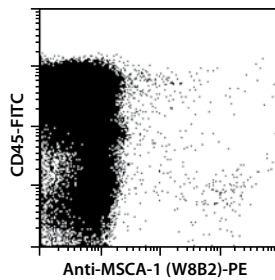
▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

7. Wash cells by adding 1–2 mL of buffer per 10<sup>7</sup> cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
8. (Optional) If Anti-MSCA-1 (W8B2)-Biotin was used, resuspend the cell pellet in 100 μL of buffer, add 10 μL of anti-biotin antibody (Anti-Biotin-FITC, Anti-Biotin-PE, or Anti-Biotin-APC), and continue as described in steps 5 and 6.
9. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

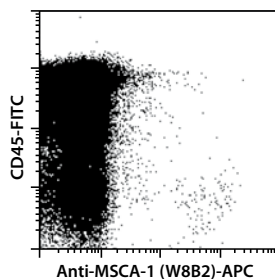
### 3. Examples of immunofluorescent staining with Anti-MSCA-1 (W8B2) antibodies

Human bone marrow mononuclear cells (BM MNCs) were stained with Anti-MSCA-1 (W8B2) antibodies conjugated to PE (a) or APC (b) as well as CD45-FITC (# 130-080-202), and analyzed by flow cytometry. Cells stained with Anti-MSCA-1 (W8B2)-Biotin (c) were stained with Anti-Biotin-APC (# 130-090-856) as well as CD45-FITC. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

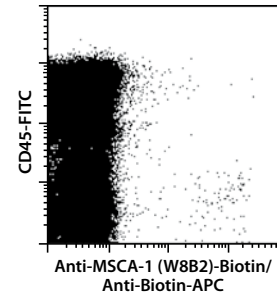
(a) Human BM MNCs stained with Anti-MSCA-1 (W8B2)-PE.



(b) Human BM MNCs stained with Anti-MSCA-1 (W8B2)-APC.



(c) Human BM MNCs stained with Anti-MSCA-1 (W8B2)-Biotin, Anti-Biotin-APC, and CD45-FITC.



### 4. References

1. Bühring, H. J. *et al.* (2007) Novel markers for the prospective isolation of human MSC. *Ann. N. Y. Acad. Sci.* 1106: 262–271.
2. Battula, V. L. *et al.* (2007) Human placenta and bone marrow derived MSC cultured in serum-free, b-FGF-containing medium express cell surface frizzled-9 and SSEA-4 and give rise to multilineage differentiation. *Differentiation* 75: 279–291.

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

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.

MACS is a registered trademark and autoMACS is a trademark of Miltenyi Biotec GmbH.

Copyright © 2008 Miltenyi Biotec GmbH. All rights reserved.