Miltenyi Biotec

Anti-MSCA-1 (W8B2) MicroBead Kit
human

Order no. 130-093-583

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1. Description

Components	**2 mL Anti-MSCA-1 (W8B2) MicroBeads, human:** MicroBeads conjugated to monoclonal Anti-MSCA-1 (W8B2) antibodies (isotype: mouse IgG1). **2 mL FcR Blocking Reagent, human:** Human IgG.
Capacity	For 10^9 total cells, up to 100 separations.
Product format	Anti-MSCA-1 (W8B2) MicroBeads are supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Principle of the MACS® Separation

First, the MSCA-1$^+$ cells are magnetically labeled with Anti-MSCA-1 (W8B2) MicroBeads. Then, the cell suspension is loaded onto a MACS® Column, which is placed in the magnetic field of a MACS Separator. The magnetically labeled MSCA-1$^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of MSCA-1$^+$ cells. After removing the column from the magnetic field, the magnetically retained MSCA-1$^+$ cells can be eluted as the positively selected cell fraction. To increase the purity, the positively selected cell fraction containing the MSCA-1$^+$ cells is separated over a second column.

1.2 Background information

The Anti-MSCA-1 (W8B2) MicroBead Kit, based on the antibody clone W8B2, has been developed for the isolation of mesenchymal stromal cells (MSCs) from bone marrow aspirates. The Anti-MSCA-1 (W8B2) MicroBeads recognize the mesenchymal stromal cell antigen 1 (MSCA-1), a so far unknown antigen. The kit contains all necessary reagents for the magnetic isolation of MSCA-1$^+$ cells, including Anti-MSCA-1 (W8B2) MicroBeads for magnetic labeling and FcR Blocking Reagent to block non-specific labeling via Fc receptors. MSCA-1 was shown to be restricted to MSCs in the CD271^{bright} population.[1] These CD271^{bright}CD45^{dim} MSCs have a much higher clonogenic capacity compared to the CD271$^+$CD45$^+$ fraction in bone marrow. Therefore, MSCA-1 is the best suitable marker to isolate MSCs with a high proliferative potential from bone marrow. MSCA-1 expression was not found on placenta-derived MSCs after culture.[2] For the efficient isolation as well as optimized expansion of MSCA-1$^+$ MSCs, the MSC Research Tool Box – MSCA-1 (W8B2) is available containing the Anti-MSCA-1 (W8B2) MicroBead Kit, NH Expansion Medium, and CytoMix – MSC, a cytokine mix optimized for the expansion of isolated MSCs.

1.3 Applications

- Positive selection of MSCs with a high proliferative capacity from bone marrow aspirates.
- Positive selection of MSCs with a high proliferative capacity from other tissues, such as lipoaspirate.

1.4 Reagent and instrument 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). Degas buffer before use, as air bubbles could block the column.

  ▲ **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 bovine serum. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- MACS Columns and MACS Separators: MSCA-1$^+$ cells can be enriched by using MS, LS, or XS Columns. Positive selection can also be performed by using the autoMACS or the autoMACS Pro Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Positive selection			
MS	10^7	2×10^8	MiniMACS, OctoMACS, VarioMACS, SuperMACS
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
XS	10^9	2×10^{10}	SuperMACS
autoMACS	2×10^8	4×10^9	autoMACS, autoMACS Pro

▲ **Note:** Column adapters are required to insert certain columns into the VarioMACS™ or SuperMACS™ Separators. For details see the respective MACS Separator data sheet.

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- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., MSCA-1 (W8B2)-PE, MSCA-1 (W8B2)-APC, CD45-FITC, CD45-PE, CD45-APC, CD45-VioBlue®, CD271 (LNGFR)-PE, CD271 (LNGFR)-APC. For more information about other fluorochrome conjugates see www.miltenyibiotec.com.
- (Optional) MACS Nonhematopoietic Stem Cell Media, for example, NH CFU-F Medium (# 130-091-676), NH Expansion Medium (# 130-091-680), NH AdipoDiff Medium (# 130-091-677), NH ChondroDiff Medium (# 130-091-679), or NH OsteoDiff Medium (# 130-091-678).
- (Optional) Propidium iodide (PI) or 7-AAD for flow cytometric exclusion of dead cells.
- (Optional) Dead Cell Removal Kit (# 130-090-101) for the depletion of dead cells.
- (Optional) Pre-Separation Filters (# 130-041-407) to remove cell clumps.

2. Protocol

2.1 Sample preparation

▲ Dead cells may bind non-specifically to MACS MicroBeads. To remove dead cells, we recommend using density gradient centrifugation or the Dead Cell Removal Kit (# 130-090-101).

▲ Use fresh bone marrow only. Avoid freezing and thawing of bone marrow cells and perform all of the following steps under sterile conditions in a laminar flow hood.

2.1.1 Preparation of human bone marrow mononuclear cells (BM MNCs)

1. Dilute aspirated human bone marrow at a ratio of 6:1 with sterile PBS containing 2 mM EDTA (PBS/EDTA buffer), for example, dilute 30 mL bone marrow with 5 mL PBS/EDTA buffer to a final volume of 35 mL.
2. Pass cells through a 100 µm membrane filter to remove bone fragments and fat clumps.
   ▲ **Note:** Wet filter with PBS or PBS/EDTA buffer before use.
3. Carefully layer 35 mL of diluted cell suspension over 15 mL of Ficoll-Paque PLUS in a 50 mL conical tube.
4. Centrifuge at 445×g for 35 minutes at room temperature in a swing bucket rotor without brake.
5. Aspirate the upper layer leaving the mononuclear cell layer undisturbed at the interface.
6. Carefully transfer the bone marrow mononuclear cells (BM MNCs) at the interface to a new 50 mL conical tube.
7. Wash cells by adding up to 40 mL of PBS/EDTA buffer, mix gently and centrifuge for 10 minutes at 300×g at room temperature.
8. Aspirate supernatant completely.
9. Repeat steps 7 and 8.
10. Resuspend cells in 2 mL of PBS/EDTA buffer.
11. Determine cell number and viability using a hemocytometer by Trypan Blue exclusion.



2.2 Magnetic labeling

▲ Work fast, keep cells cold, and use pre-cooled solutions. This will prevent capping of antibodies on the cell surface and non-specific cell labeling.

▲ Volumes for magnetic 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).

▲ **Note:** For subsequent cell culture of MSCA-1 $(W8B2)^+$ cells, start with at least 5×10^7 BM MNCs and scale up all reagent volumes and total volumes accordingly.

▲ For optimal performance it is important to obtain a single-cell suspension before magnetic separation. Pass cells through 30 µm nylon mesh (Pre-Separation Filters, # 130-041-407) to remove cell clumps which may clog the column. Wet filter with buffer before use.

▲ The recommended incubation temperature is 2–8 °C. Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 60 µL of buffer per 10^7 total cells.
4. Add 20 µL of FcR Blocking Reagent and 20 µL of Anti-MSCA-1 MicroBeads per 10^7 total cells.
5. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
6. (Optional) Add staining antibodies, e.g., 10 µL of MSCA-1 (W8B2)-APC, and incubate for 5 minutes in the dark in the refrigerator (2–8 °C).
7. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
8. Resuspend up to 10^8 cells in 500 µL of buffer.
   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
9. Proceed to magnetic separation (2.3).



2.3 Magnetic separation

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of MSCA-1 $(W8B2)^+$ cells. For details see table in section 1.4.

Magnetic separation with MS or LS Columns

▲ To achieve highest purities, perform two consecutive column runs.

1. Place column in the magnetic field of a suitable MACS Separator. For details see the respective MACS Column data sheet.

2. Prepare column by rinsing with the appropriate amount of buffer:
   MS: 500 µL LS: 3 mL
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with the appropriate amount of buffer. Collect total effluent; this is the unlabeled cell fraction. Perform washing steps by adding buffer three times. Only add new buffer when the column reservoir is empty.
   MS: 3×500 µL LS: 3×3 mL
5. Remove column from the separator and place it on a suitable collection tube.
   ▲ **Note:** To perform a second column run, you may elute the cells directly from the first onto the second, equilibrated column instead of a collection tube.
6. Pipette the appropriate amount of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.
   MS: 1 mL LS: 5 mL
7. To increase the purity of MSCA-1 (W8B2)$^+$ cells, the eluted fraction can be enriched over a second MS or LS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

Magnetic separation with XS Columns

For instructions on the column assembly and the separation refer to the XS Column data sheet.

Magnetic separation with the autoMACS™ Separator or the autoMACS™ Pro Separator

▲ Refer to the respective user manual for instructions on how to use the autoMACS™ Separator or the autoMACS Pro Separator.

▲ Buffers used for operating the autoMACS Separator or the autoMACS Pro Separator should have a temperature of ≥ 10 °C.

▲ Program choice depends on the isolation strategy, the strength of magnetic labeling, and the frequency of magnetically labeled cells. For details refer to the section describing the cell separation programs in the respective user manual. Program recommendations below refer to separation of human bone marrow mononuclear cells (BM MNCs).

Magnetic separation with the autoMACS™ Separator

1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube at the uptake port and the fraction collection tubes at port neg1 and port pos2.
3. For a standard separation choose one of the following programs:

   Positive selection: "Posseld2"
   Collect positive fraction from outlet port pos2.

   Depletion: "Depletes"
   Collect negative fraction from outlet port neg1.

Magnetic separation with the autoMACS™ Pro Separator

1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and the fraction collection tubes in rows B and C.
3. For a standard separation choose one of the following programs:

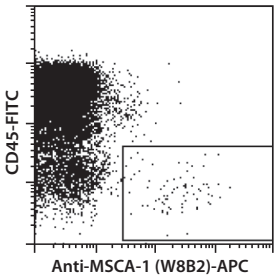
   Positive selection: "Posseld2"
   Collect positive fraction in row C of the tube rack.

   Depletion: "Depletes"
   Collect negative fraction in row B of the tube rack.

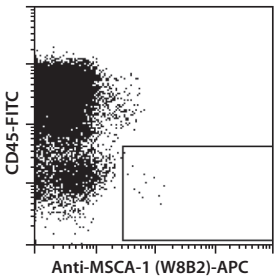
3. Example of a separation using the Anti-MSCA-1 (W8B2) MicroBead Kit

MSCA-1$^+$ cells were isolated from human bone marrow mononuclear cells (BM MNCs) using the Anti-MSCA-1 (W8B2) MicroBead Kit, two MS Columns, and a MiniMACS™ Separator. Cells are fluorescently stained with Anti-MSCA-1 (W8B2)-APC and CD45-FITC. Cell debris and dead cells are excluded from the analysis based on scatter signals and PI fluorescence.

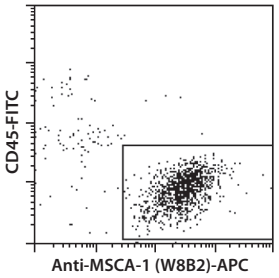
Before separation



MSCA-1$^-$ cells



MSCA-1$^+$ cells



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.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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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