Miltenyi Biotec

Cardiac Progenitor Isolation Kit (Sca-1)
mouse

Order no. 130-098-374

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1. Description

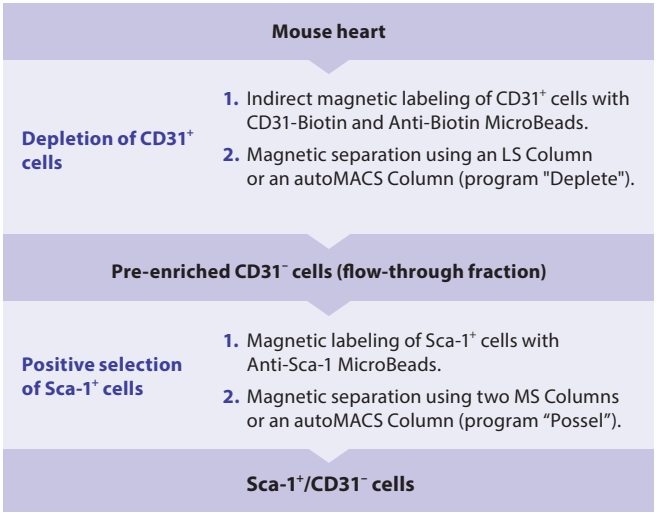
Components	**1 mL CD31-Biotin:** biotin-conjugated monoclonal anti-mouse antibodies against CD31. **2 mL Anti-Biotin MicroBeads:** MicroBeads conjugated to monoclonal anti-biotin antibody. **1 mL Anti-Sca-1 MicroBeads, mouse:** MicroBeads conjugated to monoclonal anti-Sca-1 antibody.
Capacity	For 100 separations.
Product format	All components 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

The isolation of Sca-1$^+$/CD31$^-$ cells is performed in a two-step procedure. First, the CD31$^+$ cells are indirectly magnetically labeled with CD31-Biotin, as primary labeling reagent, and anti-biotin monoclonal antibodies conjugated to MicroBeads, as secondary labeling reagent. The labeled cells are subsequently depleted by separation over a MACS® Column, which is placed in the magnetic field of a MACS Separator.

In the second step, the Sca-1$^+$cells are labeled with Anti-Sca-1 MicroBeads and isolated by positive selection from the pre-enriched CD31$^-$ cell fraction by separation over a MACS Column, which is placed in the magnetic field of a MACS Separator.

After removing the column from the magnetic field, the magnetically retained Sca-1$^+$/CD31$^-$ cells can be eluted as the positively selected cell fraction.



1.2 Background information

A population of cardiac progenitor cells from the adult myocardium based on their expression of stem cell antigen-1 (Sca-1) has been identified.[1] These cells express most cardiogenic transcription factors but lack expression of cardiac structural genes. After treatment with 5-azacytidine, cardiac differentiation was induced in Sca-1$^+$ cells *in vitro*. In addition, this population homed to the injured myocardium upon grafting in an ischemia/reperfusion mouse model following by cardiac differentiation and fusion to the host myocardium. The majority of Sca-1$^+$ cells in the adult mouse heart co-express the endothelial marker CD31.[1,2] It has been shown that only the CD31$^-$ but not CD31$^+$ population exhibit functional cardiomyogenic differentiation. [2] In conclusion, Sca-1$^+$/CD31$^-$ cells represent a distinct cardiac progenitor cell population, capable of differentiation into mature cardiomyocytes.

1.3 Applications

- Isolation of Sca-1$^+$/CD31$^-$ cells from dissociated murine heart tissue for further downstream analysis or cell culture.

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 mouse serum albumin, mouse serum, or fetal bovine serum (FBS). Buffers or media containing $Ca2^+$ or $Mg2^+$ are not recommended for use.

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macsde@miltenyi.com
www.miltenyibiotec.com

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- MACS Columns and MACS Separators: Depletion of CD31$^+$ cells can be performed on an LS Column. The subsequent positive selection of Sca-1$^+$ cells can be performed on an MS Column. Positive selection and depletion can also be performed by using the autoMACS Pro or the autoMACS Separator.

Column	Max. number of dissociated mouse hearts per column	Separator
Depletion		
LS	up to 10	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
Positive selection		
MS	up to 10	MiniMACS, OctoMACS, VarioMACS, SuperMACS II
Positive selection or depletion		
autoMACS	up to 10	autoMACS Pro, autoMACS

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

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., Anti-Biotin-FITC (# 130-090-857), Anti-Sca-1-APC (# 130-093-223), or CD31-PE (# 130-097-422). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) 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, 30 µm (# 130-041-407) to remove cell clumps.

2. Protocol

2.1 Sample preparation

Prepare a single-cell suspension from murine heart tissue using manual methods or the gentleMACS™ Dissociator.

For details refer to the protocols section at www.miltenyibiotec.com/protocols.

▲ 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).



2.2 Magnetic labeling of CD31$^+$ cells

▲ 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 one dissociated mouse heart (80–115 mg). When working with higher sample volumes, scale up all reagent volumes and total volumes accordingly (e.g. for two dissociated mouse hearts, use twice the volume of all indicated reagent volumes and total volumes).

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

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

1. Centrifuge cell suspension at 300×g for 7 minutes. Aspirate supernatant completely.
2. Resuspend cell pellet in 100 µL of buffer per dissociated mouse heart.
3. Add 10 µL of CD31-Biotin per dissociated mouse heart.
4. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
5. Wash cells by adding 1 mL of buffer for up to 5 dissociated mouse hearts and centrifuge at 300×g for 7 minutes. Aspirate supernatant completely.
6. Resuspend cell pellet in 80 µL of buffer per dissociated mouse heart.
7. Add 20 µL of Anti-Biotin MicroBeads per dissociated mouse heart.
8. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
9. (Optional) Wash cells by adding 1 mL of buffer per dissociated mouse heart and centrifuge at 300×g for 7 minutes. Aspirate supernatant completely.
10. Resuspend cells from up to 5 dissociated mouse hearts in 500 µL of buffer.
11. Proceed to magnetic separation (2.3).



2.3 Magnetic separation: Depletion of CD31$^+$ cells

▲ Always wait until the column reservoir is empty before proceeding to the next step.

Depletion with LS Columns

1. Place LS Column in the magnetic field of a suitable MACS Separator. For details refer to the LS Column data sheet.
2. Prepare column by rinsing with 3 mL of buffer.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with 3×3 mL of buffer. Collect total effluent; this is the unlabeled CD31$^+$ depleted cell fraction. Perform washing steps by adding buffer three times. Only add new buffer when the column reservoir is empty.
5. Proceed to 2.4 for the labeling of Sca-1$^+$ cells.

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Depletion with the autoMACS® Pro Separator or the autoMACS® Separator

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

▲ Buffers used for operating the autoMACS Pro Separator or the autoMACS 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 dissociated mouse heart tissue.

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 the following program:

   Depletion: Deplete
   Collect negative fraction in row B of the tube rack.
4. Proceed to 2.4 for the labeling of Sca-1$^+$ cells.

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 pos1.
3. For a standard separation choose the following program:

   Depletion: Deplete
   Collect negative fraction from outlet port neg1.
4. Proceed to 2.4 for the labeling of Sca-1$^+$ cells.



2.4 Magnetic labeling of Sca-1$^+$ cells

▲ 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 one dissociated mouse heart (80–115 mg). When working with higher sample volumes, scale up all reagent volumes and total volumes accordingly (e.g. for two dissociated mouse hearts, use twice the volume of all indicated reagent volumes and total volumes).

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

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

1. Centrifuge cell suspension at 300×g for 7 minutes. Aspirate supernatant completely.
2. Resuspend cell pellet in 90 µL of buffer per dissociated mouse heart.
3. Add 10 µL of Anti-Sca-1 MicroBeads per dissociated mouse heart.
4. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
5. Wash cells by adding 1 mL of buffer and centrifuge at 300×g for 7 minutes. Aspirate supernatant completely.
6. Resuspend cells from up to 5 dissociated mouse hearts in 500 µL of buffer.
7. Proceed to magnetic separation (2.5).



2.5 Magnetic separation: Positive selection of Sca-1$^+$ cells

▲ Always wait until the column reservoir is empty before proceeding to the next step.

Positive selection with MS Columns

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

1. Place MS Column in the magnetic field of a suitable MACS Separator. For details refer to MS Column data sheet.
2. Prepare column by rinsing with 500 µL of buffer.
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.
4. Wash column with 3×500 µL of buffer. Collect unlabeled cells that pass through and combine with the effluent from step 3.

   ▲ **Note:** Perform washing steps by adding buffer aliquots only when the column reservoir is empty.
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 1 mL of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.
7. To increase purity of Sca-1$^+$ cells, the eluted fraction must be enriched over a second MS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

Positive selection with the autoMACS® Pro Separator or the autoMACS® Separator

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 the following program:

   Positive selection: Possel
   Collect positive fraction in row C of the tube rack. This is the enriched Sca-1$^+$ cell fraction.

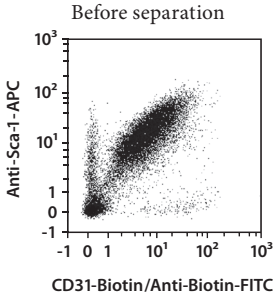
Magnetic separation with the autoMACS® Separator

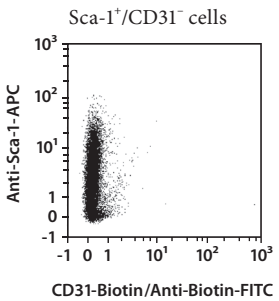
1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube at the uptake port and the fraction collection tubes at port neg1 and port pos2.
3. For a standard separation choose the following program:

   Positive selection: Possel
   Collect positive fraction from outlet port pos2. This is the enriched Sca-1$^+$ cell fraction.

3. Example of a separation using the Cardiac Progenitor Isolation Kit (Sca-1)

Sca-1$^+$/CD31$^-$ cardiac progenitor cells were isolated from dissociated mouse heart tissue by using the Cardiac Progenitor Isolation Kit (Sca-1), an LS and an MS Column, a MidiMACS™ and a MiniMACS™ Separator. The cells were fluorescently stained with Anti-Biotin-FITC (# 130-090-857) and Anti-Sca-1-APC (# 130-093-223) and analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.





4. References

1. Oh, H. *et al.* (2003) Cardiac progenitor cells from adult myocardium: Homing, differentiation, and fusion after infarction. Proc. Natl. Acad. Sci. 100: 12313–12318.
2. Pfister, O. *et al.* (2005) CD31$^-$ but not CD31$^+$ cardiac side population cells exhibit functional cardiomyogenic differentiation. Circ. Res. 97: 52–61.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com/local to find your nearest Miltenyi Biotec contact.

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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