Miltenyi Biotec

Anti-SSEA-1 (CD15) MicroBeads
human and mouse

Order no. 130-094-530

Contents

1. Description

Components	2 mL Anti-SSEA-1 (CD15) MicroBeads, human and mouse: MicroBeads conjugated to monoclonal anti-mouse CD15 antibodies (isotype: mouse IgM).
Capacity	For 10^9 total cells, up to 100 separations.
Product format	Anti-SSEA-1 (CD15) 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 Anti-SSEA-1 $(CD15)^+$ cells are magnetically labeled with Anti-SSEA-1 (CD15) 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 Anti-SSEA-1 $(CD15)^+$ cells are retained within the column. The unlabeled cells run through. After removing the column from the magnetic field, the magnetically retained Anti-SSEA-1 $(CD15)^+$ cells can be eluted as the positively selected cell fraction.

1.2 Background information

SSEA-1 is one of the hallmark mouse embryonic stem cell (ESC) surface markers and is associated with the state of pluripotency. SSEA-1 is known to be expressed on undifferentiated mouse ESCs[1-3] and on mouse induced pluripotent stem (iPS) cells[4]. During the reprogramming of mouse fibroblasts to iPS cells, SSEA-1 is one of the first pluripotency markers that is expressed.[5] In contrast to that, SSEA-1 is not expressed on undifferentiated human ESCs but on spontaneously differentiated human ESCs.[6] The Anti-SSEA-1 (CD15) MicroBeads bind to the carbohydrate antigen 3-fucosyl-N-acetyl-lactosamine also called CD15 or Lewis x.

1.3 Applications

- Isolation of mouse ESCs or iPSCs from Feeder cells.
- Removal of unwanted remaining pluripotent cells in mouse ESC- or iPSC-derived differentiation cultures.
- Enrichment of reprogrammed mouse iPS cells.
- Depletion of spontaneously differentiated cells from human ESC or iPSC cultures.

1.4 Reagent and instrument requirements

- Buffer: Dulbecco's phosphate-buffered saline (DPBS) without Ca^{2+} and Mg^{2+}, with 0.5% bovine serum albumin (BSA), and 2 mM EDTA. 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 or mouse serum albumin, human or mouse serum, or fetal bovine serum (FBS). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- MACS Columns and MACS Separators: For optimal recovery the use of an LS Column is recommended. Anti-SSEA-1 $(CD15)^+$ cells can also be enriched by using MS or XS Columns or depleted with the use of LD, CS, or D Columns. Positive selection or depletion can also be performed by using the autoMACS Pro or the autoMACS 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
Depletion			
LD	10^8	5×10^8	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
CS	2×10^8		VarioMACS, SuperMACS
D	10^9		SuperMACS
Positive selection or depletion			
autoMACS	2×10^8	4×10^9	autoMACS Pro, autoMACS

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

- Trypsin solution: 0.05% trypsin, 2 mM EDTA.
- Culture medium.

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- (Optional) Fluorochrome-conjugated CD15 antibodies for flow cytometric analysis, e.g., CD15-PE, human (# 130-091-375, cross-reactive with mouse). For more information about fluorochrome-conjugated antibodies see www.miltenyibiotec.com.
- (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 (# 130-041-407) to remove cell clumps.

2. Protocol

2.1 Sample preparation

Isolation of mouse ESCs or iPSCs from Feeder cells

1. Remove culture medium and wash culture plates twice with DPBS.
2. Trypsinize with 2 mL of trypsin solution per 10 cm culture dish for 5 minutes at 37 °C.
3. Stop enzymatic reaction by addition of 8 mL of culture medium containing FBS or trypsin inhibitor.
4. Dissociate to single-cell suspension by pipetting up and down using a 10 ml serological pipette.

Removal of unwanted remaining pluripotent cells in mouse ESC- or iPSC-derived differentiation cultures

Differentiation cultures may be composed of a variety of different cell types and extracelllular matrix components depending on the differentiation technique, for example, embryoid body (3D) or monolayer (2D) differentiation, and direction, such as endo-, meso- or ectodermal differentiation. These differences require optimized protocols for single cell preparation and require optimization by testing suited enzymes or enzyme mixes, for example, trypsin, accutase, collagenases, that give single-cell suspension with high viability. Therefore, pre-testing of different strategies is strongly recommended in advance of experiments using MACS Technology.

Enrichment of reprogrammed mouse iPSCs

Single-cell suspensions from cell cultures in the process of reprogramming have been obtained by incubation in trypsin solution (0.25% trypsin, 1 mM EDTA) for 5 minutes at 37 °C, and repetitive pipetting and transfer through a 40 µm cell strainer.[4]

Depletion of spontaneously differentiated cells from human ESC or iPSC cultures

Human embryonic stem cells (hESCs) or human induced pluripotent stem cells (iPSCs) are sensitive to singling and some precautions have to be taken in order to make cells amenable to single-cell dissociation. Please make sure to establish a suited method for single-cell passaging before conducting experiments using the MACS Technology to ensure that the hESCs/iPSCs can be propagated at high viability under these conditions. Different protocols have been described to obtain single-cell suspensions of hESCs/iPSCs, for example, by using trypsin or accutase[7,8]. The use of a ROCK-specific inhibitor as a medium component during subculturing might be beneficial in order to obtain highly viable hESCs/iPSCs after single-cell dissociation and separations using MACS Technology[9].

▲ 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

▲ The following protocol has been optimized for the isolation of mouse embryonic stem cells (ESCs) from fibroblasts.

▲ 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).

▲ 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, # 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. Working on ice is not recommended. 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 80 µL of buffer per 10^7 total cells.
4. Add 20 µL of Anti-SSEA-1 (CD15) MicroBeads per 10^7 total cells.
5. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
6. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. Resuspend up to 10^8 cells in 500 µL of buffer.

   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.

   ▲ **Note:** For depletion with LD Columns, resuspend up to 1.25×10^8 cells in 500 µL of buffer.
8. (Optional) Collect an aliquot of up to 10^6 cells and adjust to 100 µL with buffer. Add staining antibodies, e.g., 10 µL of CD15-PE (# 130-091-375), and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).
9. Proceed to magnetic separation (2.3).

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2.3 Magnetic separation

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of Anti-SSEA-1 (CD15)$^+$ cells. For optimal recovery the use of an LS Column is recommended. For details see table in section 1.4.

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

Magnetic separation with MS or LS Columns

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. Collect flow-through containing unlabeled cells.
4. Wash column with the appropriate amount of buffer. Collect unlabeled cells that pass through and combine with the effluent from step 3.
   MS: 3×500 µL **LS: 3×3 mL**

   ▲ **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.
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**

Magnetic separation with XS Columns

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

Depletion with LD Columns

1. Place LD Column in the magnetic field of a suitable MACS Separator. For details see LD Column data sheet.
2. Prepare column by rinsing with 2 mL of buffer.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with 2×1 mL of buffer. Collect total effluent; this is the unlabeled cell fraction. Perform washing steps by adding buffer two times. Only add new buffer when the column reservoir is empty.

Depletion with CS Columns

1. Assemble CS Column and place it in the magnetic field of a suitable MACS Separator. For details see CS Column data sheet.
2. Prepare column by filling and rinsing with 60 mL of buffer. Attach a 22G flow resistor to the 3-way stopcock of the assembled column. For details see CS Column data sheet.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with 30 mL buffer from the top. Collect total effluent; this is the unlabeled cell fraction.

Depletion with D Columns

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

Magnetic separation 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.

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.

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

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:

   Positive selection: "Possel"
   Collect positive fraction from outlet port pos1.

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

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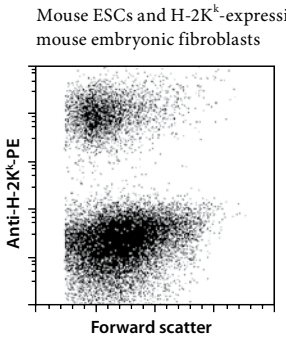
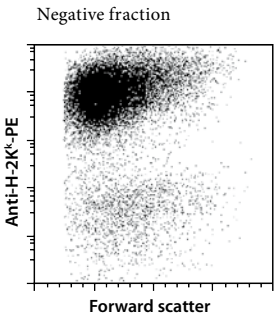
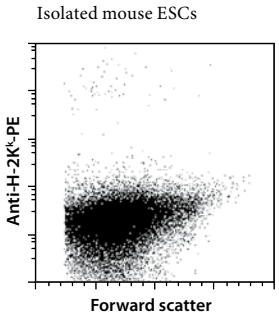
3. Example of a separation using the Anti-SSEA-1 (CD15) MicroBeads

Isolation of mouse embryonic stem cells (ESCs) from a mixture of mouse ESCs and H-2Kk–expressing transgenic mouse embryonic fibroblasts (MEFs) using Anti-SSEA-1 (CD15) MicroBeads, an LS Column, and a MidiMACS™ Separator. Cells were stained with anti-H-2K^k-PE and analyzed by flow cytometry. Expression of ectopic H-2K^k was performed solely as a means of identifying MEFs within mixed cultures and is not required for magnetic separation. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.







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All protocols and data sheets are available at **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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