Miltenyi Biotec

Anti-SSEA-4 MicroBeads
human

Order no. 130-097-855

Contents

1. Description

Components	2 mL Anti-SSEA-4 MicroBeads, human: MicroBeads conjugated to monoclonal anti-human SSEA-4 antibodies.
Capacity	For 10^9 total cells, up to 100 separations.
Product format	Anti-SSEA-4 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 SSEA-4$^+$ cells are magnetically labeled with Anti-SSEA-4 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 SSEA-4$^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of SSEA-4$^+$ cells. After removing the column from the magnetic field, the magnetically retained SSEA-4$^+$ cells can be eluted as the positively selected cell fraction.

1.2 Background information

Anti-SSEA-4 MicroBeads have been developed for the separation of cells based on the presence of the carbohydrate epitope stage specific embryonic antigen 4 (SSEA-4). SSEA-4 is found on undifferentiated human embryonic stem (ES) cells[1], induced pluripotent (iPS) cells[2], embryonal carcinoma (EC) cells[3], and embryonic germ (EG) cells[4] as well as a variety of somatic stem cells, such as dental pulp stem cells, umbilical cord blood-derived very small embryonic like stem cells (VSELs)[5], and mesenchymal stromal cells (MSCs)[6].

1.3 Applications

- Positive selection or depletion of cells expressing the SSEA-4 antigen.

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.
- MACS Columns and MACS Separators: SSEA-4$^+$ cells can be enriched by using LS Columns or depleted with the use of LD 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			
LS	2×10^7	4×10^7	MidiMACS, QuadroMACS, SuperMACS II
Depletion			
LD	1.5×10^7	3×10^7	MidiMACS, QuadroMACS, SuperMACS II
Positive selection or depletion			
autoMACS	5×10^7	10^8	autoMACS Pro, autoMACS

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

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., Labeling Check Reagent-FITC (# 130-095-226) to detect MicroBeads and CD326 (EpCAM)-APC. 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) Pre-Separation Filters, 30 µm (# 130-041-407) to remove cell clumps.

Additional requirements for harvesting of ES or iPS cells (refer to protocol 2.2)

- Trypsin solution: 0.05% trypsin, 2 mM EDTA or CellMates™ Accutase® Cell Detachment Solution (# 130-095-545).
- Culture medium

  ▲ **Note:** If human ES and iPS cells are further cultivated after the separation the magnetic labeling and the separation should be performed in standard culture medium.
- (Optional) hES Cell Cloning & Recovery Supplement (# 130-095-690).

  ▲ **Note:** For subculturing, supplementation of the culture medium with 2 µM Thiazovivin (hES Cell Cloning & Recovery Supplement) is strongly recommended for the first 2 days of cultivation.

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2. Protocol

2.1 Sample preparation

When working with tissues or blood, prepare a single-cell suspension using standard methods.

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

When working with human embryonic stem cells (ES) or induced pluripotent cells (iPS) refer to section 2.2.

2.2 Harvesting of human embryonic stem cells (ES) or induced pluripotent cells (iPS)

Human ES or iPS cells are sensitive to singling and some precautions have to be taken in order to make cells amenable to single-cell dissociation.
Different protocols have been described to obtain single-cell suspensions of human ES and iPS cells, for example, by using trypsin or accutase.[8,9] If cells are further cultivated after the separation the magnetic labeling and separation should be performed in standard culture medium. The use of ROCK inhibitors as medium components is beneficial in order to obtain highly viable hESCs/iPSCs after single-cell dissociation.[10] We strongly recommend using the hES Cell Cloning & Recovery Supplement (# 130-095-690) containing the active component Thiazovivin.[11,12]

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.
   ▲ **Note:** For human ESC or iPSC alternative enzymes have been succesfully employed, e.g., CellMates™ Accutase® Cell Detachment Solution (# 130-095-545).
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.
5. 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 or culture medium before use.

2.3 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).

▲ 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. Determine cell number.
2. Centrifuge cell suspension at 300×g for 5 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 80 µL of buffer or culture medium per 10^7 total cells.
   ▲ **Note:** Human ES an iPS cells should be magnetically labeled and separated in standard culture medium to achieve highest viability. However, for preparative separations the use of buffer is advisable.
4. Add 20 µL of Anti-SSEA-4 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 5 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.
8. Proceed to magnetic separation (2.3).

2.4 Magnetic separation

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of SSEA-4$^+$ cells. For details refer to the table in section 1.4.

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

Magnetic separation with LS Columns

1. Place column in the magnetic field of a suitable MACS Separator. For details refer to the respective MACS Column data sheet.
2. Prepare column by rinsing 3 mL of buffer or culture medium.
   ▲ **Note:** If human ES and iPS cells are further cultivated after the separation the magnetic labeling and the separation should be performed in standard culture medium.
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.
4. Wash column with 3×3 mL of buffer. Collect unlabeled cells that pass through and combine with the flow-through 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.
6. Pipette 5 mL of buffer or culture medium onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.
   ▲ **Note:** For subculturing of ES and iPS cells, supplementation of the culture medium with 2 µM Thiazovivin (hES Cell Cloning & Recovery Supplement, # 130-095-690) is strongly recommended for the first 2 days of cultivation.
7. (Optional) To increase the purity of SSEA-4$^+$ cells, the eluted fraction can be enriched over a second LS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

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Depletion with LD Columns

1. Place LD Column in the magnetic field of a suitable MACS Separator. For details refer to the LD Column data sheet.
2. Prepare column by rinsing with 2 mL of buffer or culture medium.
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 flow-through; this is the unlabeled cell fraction. Perform washing steps by adding buffer two times. Only add new buffer when the column reservoir is empty.

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 one of the following programs:

   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

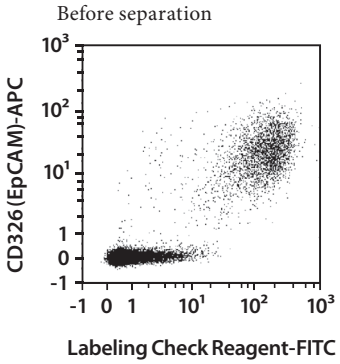
1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube at the uptake port and the fraction collection tubes at port neg1 and pos1.
3. For a standard separation choose one of the following programs:

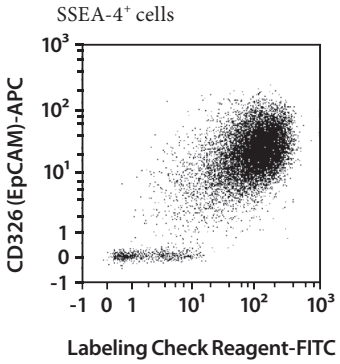
   Positive selection: Possel
   Collect positive fraction from outlet pos1.

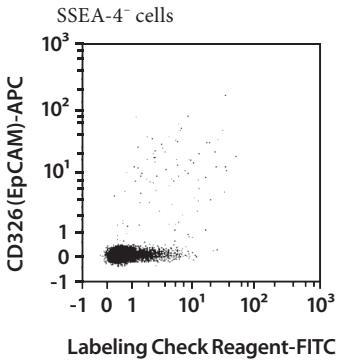
   Depletion: Depletes
   Collect negative fraction from outlet port neg1.

3. Example of a separation using Anti-SSEA-4 MicroBeads

SSEA-4 expressing cells were isolated from a heterogeneous suspension of U937 cells (SSEA-4$^-$CD326$^-$) and NTERA cells (SSEA-4$^+$CD326$^+$) using Anti-SSEA-4 MicroBeads, an LS Column, and a MidiMACS™ Separator. Cells were fluorescently stained with CD326 (EpCAM)-APC and Labeling Check Reagent-FITC (# 130-095-226), which specifically recognizes the MicroBeads, and analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris and dead cells were excluded based on scatter signals and propidium iodide fluorescence.







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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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