Miltenyi Biotec

Pluripotent Stem Cell MicroBeads
human

Order no. 130-095-804

Contents

1. Description

Components	2 mL Pluripotent Stem Cell MicroBeads, human: MicroBeads conjugated to monoclonal anti-human CD326 antibodies (isotype: mouse IgG1).
Capacity	For 2×10^8 total cells, up to 100 separations.
Product format	Pluripotent Stem Cell 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 CD326$^+$ pluripotent stem cells are magnetically labeled with Pluripotent Stem Cell 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 pluripotent stem cells are retained within the column. The unlabeled cells run through. After removing the column from the magnetic field, the magnetically retained CD326$^+$ pluripotent stem cells can be eluted as the positively selected cell fraction.

1.2 Background information

Pluripotent Stem Cell MicroBeads have been developed for the separation of human pluripotent stem cells based on the expression of the CD326 antigen (EPCAM). CD326 is expressed on undifferentiated human embryonic stem (ES) cells and induced pluripotent stem (iPS) cells and is localized to OCT4-positive pluripotent cells. Its expression is down-regulated upon differentiation.[1,2]

1.3 Applications

- Positive selection of undifferentiated CD326$^+$ pluripotent stem cells, for example, human ES and iPS cells.
- Enrichment of iPS cells after reprogramming.

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).

- Trypsin solution: 0.05% trypsin, 2 mM EDTA or CellMates™ Accutase® Cell Detachment Solution (# 130-095-545).
- MACS Columns and MACS Separators: For optimal purity and recovery the use of an LS Column is strongly recommended. Positive selection 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	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
autoMACS	2×10^8	4×10^9	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.

- Pre-Separation Filters (# 130-041-407) to remove cell clumps.
- 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.

- (Optional) Fluorochrome-conjugated CD326 antibodies for flow cytometric analysis, e.g., CD326 (EpCAM)-APC (# 130-091-254). For more information about antibodies refer to www.miltenyibiotec.com.

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2. Protocol

2.1 Sample preparation

Harvesting of human embryonic stem cells (ES) or induced pluripotent cells (iPS) cultured on Feeder cells

▲ 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 or culture medium before use.

▲ 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.[3,4] If cells are further cultivated after the separation the magnetic labeling and separation should be performend 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.[5] We strongly recommend using the hES Cell Cloning & Recovery Supplement (# 130-095-690) containing the active component Thiazovivin.[6,7]

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, # 130-041-407) to remove cell clumps which may clog the column. Moisten filter with buffer or culture medium before use.

2.2 Magnetic labeling

The following protocol has been optimized for positive selection only.

▲ 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 2×10^6 total cells. When working with fewer than 2×10^6 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 4×10^6 total cells, use twice the volume of all indicated reagent volumes and total volumes).

▲ 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 is not recommended.

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 2×10^6 total cells.

   ▲ **Note:** Human ESC an iPSC 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 Pluripotent Stem Cell MicroBeads per 2×10^6 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 CD326 (EpCAM)-APC (# 130-091-254), 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 2×10^6 cells and centrifuge at 300×g for 5 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 the autoMACS Separator or an LS Column with an appropriate MACS Separator. 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 LS Column by rinsing with 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 or culture medium. 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, 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.

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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.

Magnetic separation with the autoMACS® Separator

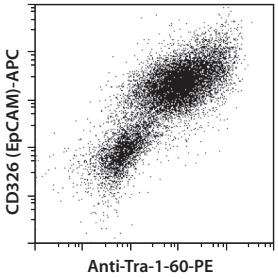
1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube at the uptake port and the fraction collection tubes at port neg1 and port pos1.
3. For a standard separation choose the following program:

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

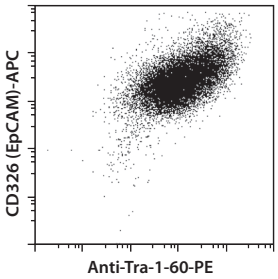
3. Example of a separation using Pluripotent Stem Cell MicroBeads

Pluripotent (CD326$^+$) iPS cells were isolated from cultures containing spontaneously differentiated iPS cells grown on mouse embryonic feeder cells using Pluripotent Stem Cell MicroBeads, an LS Column, and a MidiMACS™ Separator. Cells were fluorescently stained with CD326 (EpCAM)-APC (# 130-091-254), Stemgent® Phycoerythrin (PE) Anti-Human TRA-1-60 Antibody (# 130-095-624) and Anti-Feeder-Biotin (coming soon) as well as with Anti-Biotin-FITC (# 130-090-857) 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. Feeder cells are excluded based on staining with Anti-Feeder-Biotin.

Before separation



Enriched CD326$^+$ pluripotent stem cells



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4. References

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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/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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