Miltenyi Biotec

Feeder Removal MicroBeads
mouse

Order no. 130-095-531

Contents

1. Description

Components	2 mL Feeder Removal MicroBeads, mouse: MicroBeads conjugated to monoclonal anti-mouse feeder antibodies (isotype: rat IgG1, κ).
Capacity	For 10^9 total cells, up to 100 separations.
Product format	Feeder Removal 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 fibroblasts (e.g. mEF, NIH3T3) are magnetically labeled with the Feeder Removal 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 fibroblasts are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of fibroblasts.

1.2 Background information

The most prevalent cultivation method for maintenance of mouse and human embryonic stem (ES) cells and induced pluripotent stem (iPS) cells is co-culturing on mouse embryonic fibroblast feeder cell layers. Different feeder cell types are employed, among these primary embryonic fibroblasts (mEF) and mEF-derived cell lines (e.g. NIH3T3). Feeder cells are also used in maintenance cultures of primary keratinocytes. Depletion of feeder cells prior to differentiation or expression profiling experiments is benefical in order to guarantee reproducible results.

1.3 Applications

- Depletion of mouse primary fibroblasts from ES or iPS cell co-cultures.
- Depletion of mouse feeder cells from keratinocyte co-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).

- Culture medium

  ▲ **Note:** If human ES and iPS cells are further cultured after the separation the magnetic labeling and the separation should be performed in standard culture medium.

- Trypsin solution: 0.05% trypsin, 2 mM EDTA.
- MACS Columns and MACS Separators: For optimal purity and recovery the use of an LS Column is strongly recommended. 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
Depletion			
LS	2×10^7	4×10^7	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
autoMACS	5×10^7	10^8	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) 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) CellMates™ Accutase® Cell Detachment Solution (# 130-095-545)
- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., Anti-Feeder-PE, mouse (# 130-096-094). 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.

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2. Protocol

2.1 Sample preparation

▲ Removal of feeder cells from mouse ES or iPS cells
No special precautions have to be considered. Cells can be magnetically labeled and separated in buffer as well as standard culture medium if desired.

▲ Removal of feeder cells from human ES or iPS cells
Human embryonic stem (hES) cells or human induced pluripotent stem (iPS) cells are sensitive to singling and some precautions have to be taken in order to make cells amenable to single-cell dissociation.

Harvesting of mouse or human ES or iPS cells cultured on 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.

   ▲ **Note:** For human ES or iPS cells 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 before use.

▲ Different protocols have been described to obtain single-cell suspensions of hES and iPS cells, for example, by using trypsin or Accutase[1,2]. 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 hES and iPS cells after single-cell dissociation[3]. It is strongly recommended to use the hES Cell Cloning & Recovery Supplement (# 130-095-690) containing the active component Thiazovivin during separation and initial culture.[4,5]

2.2 Magnetic labeling

▲ The following protocol has been optimized for the isolation of mouse and human ES and iPS cells cultivated on feeder 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 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 and iPS cells should be magnetically labeled and separated in standard culture medium to achieve highest viability.
4. Add 20 µL of Feeder Removal MicroBeads per 10^7 total cells.
5. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
6. Adjust volume to 500 µL for up to 5×10^7 and to 1 mL for up to 10^8 cells using buffer or standard culture medium. Do not centrifuge!
7. Proceed to magnetic separation (2.3).

2.3 Magnetic separation

▲ 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 with 3 mL of buffer or culture medium.

   ▲ **Note:** If human ES and iPS cells are further cultured 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 (ES and iPS cells).
4. Wash column with 2×1 mL of buffer or standard medium. Collect unlabeled cells (ES and iPS cells) that pass through and combine with the flow-through from step 3.

   ▲ **Note:** Perform washing steps by adding buffer or culture medium aliquots only when the column reservoir is empty.

   ▲ **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 two days of cultivation.

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.

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▲ 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:

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

Magnetic separation with the autoMACS® Separator

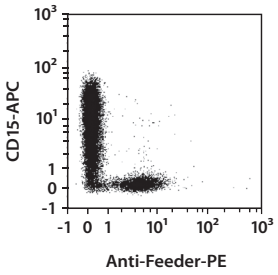
1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube at the uptake port and the fraction collection tubes at port neg1 and port pos1.
3. For a standard separation choose the following program:

   Depletion: Depletes
   Collect negative fraction from outlet port neg1.

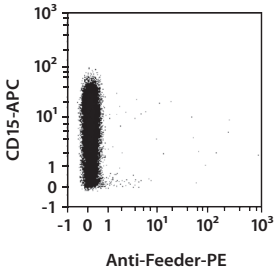
3. Example of a separation using the Feeder Removal MicroBeads

Mouse embryonic stem (mES) cells, cultured on mouse embryonic fibroblasts (mEF) were depleted of feeder cells using the the Feeder Removal MicroBeads, an LS Column, and a MidiMACS™ Separator. Cells were fluorescently stained with Anti-Feeder-PE (# 130-096-094) and, for detection of SSEA-1$^+$ mES cells, with CD15-APC, human (# 130-091-371, cross-reactive with mouse cells). Cells were analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from analysis based on scatter signals and propidium iodide fluorescence.
Dot plots indicate that a starting population of 10% mEF was completely removed. The purity of the enriched mES cells was about 99.9%.

Before separation



After separation



4. References

1. Ellerström, C. *et al.* (2007) Facilitated expansion of human embryonic stem cells by single-cell enzymatic dissociation. Stem Cells 25: 1690–1696.
2. Bajpai, R. *et al.* (2008) Efficient propagation of single cells Accutase-dissociated human embryonic stem cells. Mol. Reprod. Dev. 75: 818–827.
3. Watanabe, K. *et al.* (2007) A ROCK inhibitor permits survival of dissociated human embryonic stem cells. Nat. Biotechnol. 25: 681–686.
4. Lin, T. *et al.* (2009) A chemical platform for improved induction of human iPSCs. Nat. Methods. 6: 805–808.
5. Xu, Y. *et al.* (2010) Revealing a core signaling regulatory mechanism for pluripotent stem cell survival and self-renewal by small molecules. Proc. Nat. Acad. Sci. 107: 8129–8134.

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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