Miltenyi Biotec

Pluripotent Stem Cell Isolation Kit

mouse

Order no. 130-095-267

Contents

1. Description

Components	**1 mL Anti-Diff-PE:** monoclonal antibody conjugated to PE (isotype: rat IgG1) that recognizes early differentiated cells. **2 mL Anti-PE MicroBeads:** MicroBeads conjugated to monoclonal anti-PE antibodies (isotype: mouse IgG1).
Capacity	For 10^9 total cells, up to 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

First, early differentiated cells are indirectly magnetically labeled with Anti-Diff-PE and Anti-PE 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 early differentiated cells are retained within the column. The unlabeled pluripotent stem cells run through; this cell fraction is thus depleted of early differentiated cells.

1.2 Background information

Mouse embryonic and induced pluripotent stem cells can be maintained in a pluripotent state, for example, by cultivation in serum-containing medium supplemented with leukemia inhibitory factor (LIF). Expression of markers, such as the transcription factors Oct-4 and Nanog or the surface carbohydrate SSEA-1 (CD15), are mostly used to characterize pluripotency of mouse cells on a molecular level. Nevertheless, expression dynamics of these markers are rather low, limiting their potential to discriminate between pluripotent and early differentiated cells.
Our novel early differentiated cell surface marker is absent on pluripotent stem cells and rapidly up-regulated upon early differentiation allowing for untouched magnetic cell isolation of pluripotent stem cells.
The Pluripotent Stem Cell Isolation Kit has been developed for the depletion of early differentiated cells from feeder-free mouse pluripotent stem cell cultures.

1.3 Applications

- Depletion of early differentiated cells enables:
  - standardized pluripotent stem cell culture,
  - standardized pluripotent stem cell differentiation experiments,
  - profiling of pluripotent stem cells without contamination of differentiated cells, and
  - efficient generation of transgenic mice using pluripotent stem cells.

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 mouse serum albumin, 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 purity the use of an LD Column is strongly recommended. Early differentiated cells cannot be depleted by use of an MS or LS Column. 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
LD	10^8	5×10^8	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
autoMACS	2×10^8	4×10^9	autoMACS Pro, autoMACS

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

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

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2. Protocol

2.1 Sample preparation

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.

2.2 Magnetic labeling

▲ The following protocol has been optimized for depletion of early differentiated cells from mouse pluripotent stem cell cultures.

▲ 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. 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 100 µL of buffer per 10^7 total cells.
4. Add 10 µL of Anti-Diff-PE.
5. Mix well and incubate for 10 minutes in the dark 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 cell pellet in 80 µL of buffer per 10^7 total cells.
8. Add 20 µL of Anti-PE MicroBeads per 10^7 total cells.
9. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
10. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 5 minutes. Aspirate supernatant completely.
11. Resuspend up to 10^8 cells in 500 µL of buffer.
    ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
12. Proceed to magnetic separation (2.3).

2.3 Magnetic separation

▲ Choose the autoMACS Separator or an LD Column with an appropriate MACS Separator. For details see table in section 1.4.

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

Depletion with LD Columns

1. Place 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.
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.
4. Wash column with 2×1 mL of buffer. Collect unlabeled pluripotent 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.

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.

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: Depl05
   Collect negative fraction in row B of the tube rack. This fraction represents the enriched pluripotent 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.
3. For a standard separation choose the following program:

   Depletion: Depl05
   Collect negative fraction from outlet port neg1. This fraction represents the enriched pluripotent cells.

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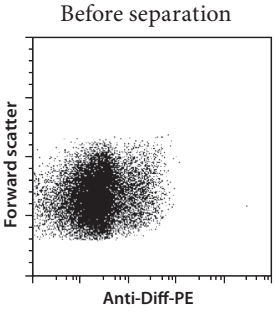
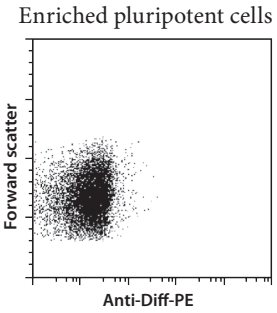
3. Example of a separation using the Pluripotent Stem Cell Isolation Kit

Early differentiated cells, which can occur during feeder-free cultivation of mouse embryonic stem cells (mESC), were depleted using the Pluripotent Stem Cell Isolation Kit, an LD Column, and a MidiMACS™ Separator. Dot Plots indicate that a starting population of 14% early differentiated cells in mESC cultures (before separation) was completely removed. The purity of the enriched pluripotent cells was about 99%. Cells were 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.





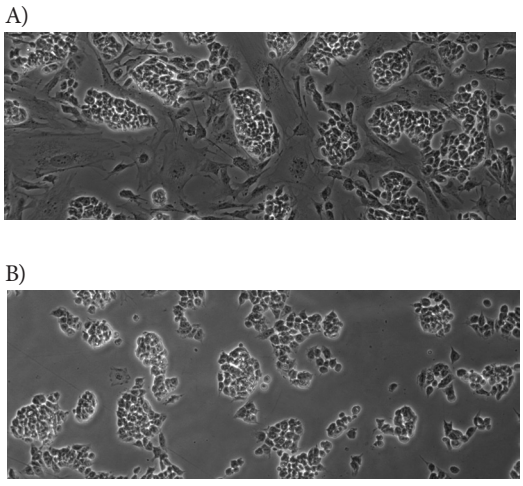


Figure 1: Phase contrast pictures of fixed feeder-free mouse embryonic stem cell cultures 1 day after plating without (A) and with separation (B) indicate efficient removal of differentiated cells.

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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