Miltenyi Biotec

# Combined removal of mouse feeder and early differentiated cells

## Contents


1. Description
   1.1 Background information
   1.2 Applications
   1.3 Reagent and instrument requirements
2. Protocol
   2.1 Sample preparation
   2.2 Magnetic labeling
   2.3 Magnetic separation
3. Reference


## 1. Description

### 1.1 Background information

Even when maintained under optimal conditions, mouse pluripotent stem cell cultures are often heterogeneous mixtures of truly pluripotent cells, feeder cells, and early differentiated cells. While early differentiated cells may still express elevated levels of SSEA-1 (CD15), they can be distinguished from truly pluripotent cells by increased expression of differentiation markers such as cytokeratin 18.

### 1.2 Applications

- Isolation of high quality pluripotent cells for sensitive downstream applications, e.g., blastocyst injection of mouse embryonic stem (ES) cells and induced pluripotent stem (iPS) cells, or tetraploid complementation.[1]

### 1.3 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.
- Trypsin solution: 0.05% trypsin, 2 mM EDTA.
- MACS® Columns and MACS Separators: Depletion can be performed by using LD Columns.

| Column | Max. number of labeled cells | Max. number of total cells | Separator |
|---|---|---|---|
| **Depletion** | | | |
| LD | $10^8$ | $5\times10^8$ | MidiMACS, QuadroMACS, VarioMACS, SuperMACS II |

▲ **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.

- Feeder Removal MicroBeads, mouse (# 130-095-531)
- Pluripotent Stem Cell Isolation Kit, mouse (# 130-095-267)
- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., Anti-Feeder-APC, mouse (# 130-096-099). 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.
- Pre-Separation Filters, 30 µm (# 130-041-407) to remove cell clumps.

## 2. Protocol

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

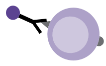
### 2.1 Sample preparation

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.
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.

SP0061


**Miltenyi Biotec GmbH**
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macs@miltenyibiotec.de
**www.miltenyibiotec.com**

**Miltenyi Biotec Inc.**
2303 Lindbergh Street, Auburn, CA 95602, USA
Phone 800 FOR MACS, +1 530 888 8871, Fax +1 530 888 8925
macs@miltenyibiotec.com



## 2.2 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 $2\times10^7$ 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 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 and 2.5 µL of Feeder Removal 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 $10^7$ 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

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

**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.
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled pluripotent cells.
4. Wash column with 2×1 mL of buffer. Collect unlabeled cells that pass through and combine with the flow-through from step 3.

# 3. Reference


1. Baskale, D. *et al.* (2012) Use of magnetically enriched pluripotent stem cells increases chimerism rate after blastocyst injection and enables the use of inbred ES cell lines for tetraploid complementation. MACS&more 14 (2).


All protocols and data sheets are available at **www.miltenyibiotec.com**.


MACS is a registered trademark and MidiMACS, QuadroMACS, SuperMACS, and VarioMACS are trademarks of Miltenyi Biotec GmbH.

Copyright © 2012 Miltenyi Biotec GmbH. All rights reserved.





Unless otherwise specifically indicated, Miltenyi Biotec products and services are for research use only and not for diagnostic or therapeutic use.