Miltenyi Biotec

Adipose Tissue Progenitor Isolation Kit
mouse

Order no. 130-106-639

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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.

1. Description

This product is for research use only.

Components	**1 mL Non-Adipocyte Progenitor Depletion Cocktail, mouse** **1 mL Adipocyte Progenitor Isolation Cocktail, mouse**
Capacity	For 50 g of tissue, up to 50 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

The isolation of adipose tissue–derived progenitor cells is performed in a two-step procedure. First, the non-target cells are directly magnetically labeled with the Non-Adipocyte Progenitor Depletion Cocktail, a cocktail of monoclonal antibodies conjugated to MACS® MicroBeads. The magnetically labeled non-target cells are depleted by retaining them within a MACS Column in the magnetic field of a MACS Separator, while the unlabeled adipose tissue progenitor cells pass through the column.
In the second step, the progenitor cells are directly labeled with the Adipocyte Progenitor Isolation Cocktail and isolated by positive selection by separation over a MACS Column, which is placed in the magnetic field of a MACS Separator.
After removing the column from the magnetic field, the magnetically retained target cells can be eluted as the positively selected cell fraction.

Mouse brown and white adipose tissue: Depletion of non-adipocyte progenitor cells

1. Direct magnetic labeling of non-target cells with the Non-Adipocyte Progenitor Depletion Cocktail.
2. Magnetic separation using an LS Column or an autoMACS Column (program "Deplete").

Pre-enriched adipocyte progenitor cell fraction (flow-through fraction):
Positive selection of adipocyte progenitor cells

1. Direct magnetic labeling of adipocyte progenitor cells with the Adipocyte Progenitor Isolation Cocktail.
2. Magnetic separation using an MS Column or an autoMACS Column (program "Posseld").

Adipocyte progenitor cells (eluted fraction)

1.2 Background information

The Adipocyte Progenitor Isolation Kit, mouse has been designed for the isolation of tissue-derived progenitor cells from dissociated brown and white adipose tissue. For optimal results, the Adipocyte Progenitor Isolation Kit, mouse should be used in combination with the Adipose Tissue Dissociation Kit, mouse and rat (# 130-105-808).

1.3 Applications

- Isolation of tissue-derived progenitor cells from mouse brown and white adipose tissue.
- Culture or direct use of enriched adipocyte progenitor cells for biochemical, physiological, pharmacological, and morphological studies.

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1.4 Reagent and instrument requirements

- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 with auto® Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C). Degas buffer before use, as air bubbles could block the column.
  Always use freshly prepared buffer. Do **not use** autoMACS Running Buffer or MACSQuant® Running Buffer as they contain a small amount of sodium azide that could affect the results.
  ▲ **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 $Ca2^+$ or $Mg2^+$ are not recommended for use.
- MACS Columns and MACS Separators: Depletion of non-target cells can be performed on an LS Column. The subsequent positive selection of adipocyte progenitor cells can be performed on a MS Column. Positive selection and depletion can also be performed by using the autoMACS Pro 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
Positive selection			
MS	10^7	2×10^7	MiniMACS, OctoMACS, VarioMACS, SuperMACS II
Positive selection or depletion			
autoMACS	2×10^7	10^8	autoMACS Pro

▲ **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) Adipose Tissue Dissociation Kit, mouse and rat (# 130-105-808) for the generation of single-cell suspension from murine adipose tissue.
- (Optional) gentleMACS™ Dissociator (# 130-093-235), gentleMACS Octo Dissociator (# 130-095-937), or gentleMACS Dissociator with Heaters (# 130-096-427)
- (Optional) gentleMACS C Tubes (# 130-093-237, # 130-096-334).
- (Optional) Basic FGF/FGF2, e.g., Human FGF-2, premium grade (# 130-093-840) for culture of adipocyte progenitor cells.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells.
- (Optional) Pre-Separation Filters, 70 µm (# 130-095-823) to remove cell clumps.

2. Protocol

2.1 Sample preparation

For preparation of single-cell suspensions from murine adipose tissue use the Adipose Tissue Dissociation Kit, mouse and rat (# 130-105-808) in combination with the gentleMACS™ Dissociators.

For details refer to www.gentleMACS.com/protocols.



2.2 Magnetic labeling of non-target 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 one gram dissociated tissue. When working with less tissue, use the same volumes as indicated. When working with higher amounts, scale up all reagent volumes and total volumes accordingly (e.g. for two gram of tissue, 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 70 µm nylon mesh (Pre-Separation Filters, 70 µm, # 130-095-823) 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. Centrifuge cell suspension at 300×g for 5 minutes. Aspirate supernatant completely.
2. Resuspend cell pellet in 80 µL of buffer per gram of tissue.
   ▲ **Note:** Always use freshly prepared buffer. For details refer to section 1.4.
3. Add 20 µL of Non-Adipocyte Progenitor Depletion Cocktail, mouse per gram of tissue.
4. Mix well and incubate for 15 minutes in the dark in the refrigerator (2–8 °C).
5. Adjust volume to 500 µL using buffer for up to 5 g of tissue.
   ▲ **Note:** If more tissue was used split the sample into multiple tubes.
6. Proceed to magnetic separation (2.3).



2.3 Magnetic separation: Depletion of non-adipocyte progenitor cells

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

Depletion with LS Columns

1. Place LS Column in the magnetic field of a suitable MACS Separator. For details refer to the LS Column data sheet.
2. Prepare column by rinsing with 3 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 total effluent and combine with the effluent from step 3; this is the unlabeled pre-enriched adipocyte progenitor cell fraction.

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

5. (Optional) Remove column from the separator and place it on a suitable collection tube. Pipette 3 mL of buffer onto the column. Immediately flush out the magnetically labeled non-progenitor cells by firmly pushing the plunger into the column.

6. Proceed to 2.4 for the labeling and enrichment of adipocyte progenitor cells.

Depletion with the autoMACS® Pro Separator

▲ Refer to the respective user manual for instructions on how to use the autoMACS® Pro Separator.

▲ Buffers used for operating the autoMACS Pro Separator should have a temperature of ≥10 °C. The autoMACS Running Buffer is inappropriate for enrichment of adipocyte progenitor cells. Use the buffer composition as descibed in section 1.4 instead.

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: Deplete
   Collect negative fraction in row B of the tube rack. This fraction represents the enriched adipocyte progenitor cells.
4. (Optional) Collect positive fraction from row C. This fraction represents the magnetically labeled non-target cells.
5. Proceed to 2.4 for the labeling and enrichment of adipocyte progenitor cells.



2.4 Magnetic labeling of adipocyte progenitor cells

▲ Volumes for magnetic labeling given below are for an initial starting volume of up to one gram dissociated tissue. For higher initial volume, scale up all volumes accordingly.

1. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
2. Resuspend cell pellet in 80 µL of buffer per gram of tissue.
3. Add 20 µL of Adipocyte Progenitor Isolation Cocktail, mouse per gram of tissue.
4. Mix well and incubate for 15 minutes in the dark in the refrigerator (2–8 °C).
5. Adjust volume to 500 µL using buffer for up to 5 g of tissue.

   ▲ **Note:** If more tissue was used split the sample into multiple tubes.
6. Proceed to magnetic separation (2.5).



2.5 Magnetic separation: Positive selection of adipocyte progenitor cells

Positive selection with MS Columns

1. Place MS Column in the magnetic field of a suitable MACS Separator. For details refer to MS Column data sheet.
2. Prepare column by rinsing with 500 µL of buffer.
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.
4. Wash column with 3×500 µL of buffer. Collect unlabeled cells that pass through and combine with the effluent 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 1 mL of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column. This fraction contains the adipocyte progenitor target cells.

Positive selection 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:

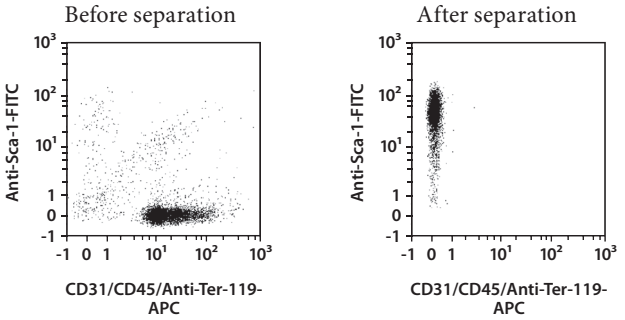
   Positive selection: Posseld
   Collect positive fraction in row C of the tube rack. This is the enriched adipocyte progenitor cell fraction.

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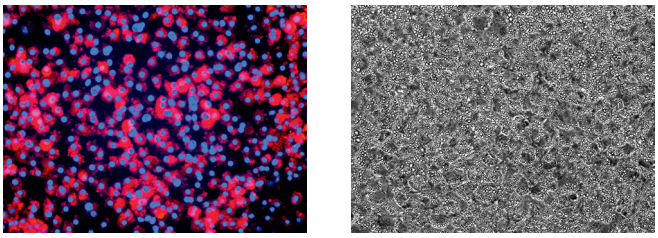
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3. Example of a separation using the Adipose Tissue Progenitor Isolation Kit

Mouse brown adipose tissue was dissociated using the Adipose Tissue Dissociation Kit, mouse and rat (# 130-105-808) in combination with the gentleMACS Octo Dissociator with Heaters. Subsequently, brown adipose tissue–derived progenitor cells were isolated using the Adipose Tissue Progenitor Isolation Kit, mouse, an LS and a MS Column, and a MidiMACS™ and a MiniMACS™ Separator. The cells were fluorescently stained with with lineage markers (CD31/CD45/Anti-Ter-119-APC) and Anti-Sca-1-FITC (# 130-102-297) 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.



In addition, the isolated adipocyte progenitor cells were cultured in expansion medium (DMEM, 10% FBS, 10 ng/mL Human FGF-2, 100 U/mL penicillin/100 U/mL streptomycin) for four days. After four days, medium was replaced with differentiation medium (DMEM, 4 nM Insulin, 1 µM Rosiglitazone, 10% FBS, 100 U/mL penicillin/100 U/mL streptomycin) and cells were incubated for three more days to induce differentiation into adipocytes. Cells were stained with Nile red to identify lipid storage and DAPI to label the nuclei.



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.

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