Miltenyi Biotec

Satellite Cell Isolation Kit
mouse

Order no. 130-104-268

Contents

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 Satellite Cell Isolation Kit, mouse: MicroBeads conjugated to a cocktail of monoclonal antibodies against non-target cells.
Capacity	For 50 g of tissue, up to 50 separations.
Product format	Cocktail is 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

Using the Satellite Cell Isolation Kit, mouse satellite cells are isolated by depletion of non-target cells. Non-target cells are directly magnetically labeled with a cocktail of monoclonal antibodies conjugated with 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 satellite cells pass through the column.

1.2 Background information

One of the most used experimental models in tissue regeneration are satellite cells. Especially the isolation and subsequent culture of satellite cells for biochemical, physiological, pharmacological, and morphological studies have a high impact in the field of tissue regeneration. The Satellite Cell Isolation Kit, mouse has been designed for the isolation of untouched satellite cells from dissociated mouse skeletal muscle. For optimal results, the Satellite Cell Isolation Kit, mouse should be used in combination with the Skeletal Muscle Dissociation Kit, mouse and rat (# 130-098-305).

1.3 Applications

- Isolation of untouched satellite cells from mouse skeletal muscle.
- Culture or direct use of enriched satellite cells for biochemical, physiological, pharmacological, and morphological studies.

1.4 Reagent and instrument requirements

- Phosphate-buffered saline (PBS)
- 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 autoMACS® 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 Ca^{2+} or Mg^{2+} are not recommended for use.
- 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 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	1×10^8	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.
- Skeletal Muscle Dissociation Kit, mouse and rat (# 130-098-305) for the generation of single-cell suspension from mouse skeletal muscle.
- gentleMACS™ Dissociator (# 130-093-235), gentleMACS Octo Dissociator (# 130-095-937), or gentleMACS Dissociator with Heaters (# 130-096-427)
- gentleMACS C Tubes (# 130-093-237, # 130-096-334)
- Red Blood Cell Lysis Solution (10×) (# 130-094-183)

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- Pre-Separation Filters, 70 µm (# 130-095-823) to remove cell clumps.
- (Optional) Anti-Integrin α-7 MicroBeads, mouse (# 130-104-261) to further increase the purity of isolated satellite cells, e.g., for direct molecular analysis, such as mRNA expression profiling.
- (Optional) Basic FGF/FGF2, e.g., Human FGF-2, premium grade (# 130-093-840) for culture of satellite cells.
- (Optional) Inside Stain Kit (# 130-090-477) to detect intracellular markers, such as Pax7.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells without fixation.

2. Protocol

2.1 Sample preparation

For preparation of single-cell suspensions from murine skeletal muscle tissue use the Skeletal Muscle Dissociation Kit, mouse and rat (# 130-098-305) in combination with the gentleMACS Dissociator and Red Blood Cell Lysis Solution (10×).

1. Dissociate mouse muscle tissue as described in the Skeletal Muscle Dissociation Kit data sheet and perform red blood cell lysis as depicted below.
2. Resuspend cell pellet in 1 mL of PBS and add 10 mL of 1× Red Blood Cell Lysis Solution to remove erythrocytes.
3. Incubate for maximal 2 minutes at room temperature (19–25 °C).
4. Centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
5. Add 15 µL of Enzyme A to 10 mL PBS in a fresh tube.
6. Resuspend cell pellet in 10 mL PBS containing Enzyme A.
7. Centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
8. Proceed to magnetic labeling (2.2).

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 1 g 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. 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.
2. Add 20 µL of Satellite Cell Isolation Kit per gram of tissue.
3. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
4. Adjust volume to 500 µL using buffer for up to 5 g of tissue. If more tissues was used split the sample into multiple tubes.
5. Proceed to magnetic separation (2.3).

2.3 Magnetic separation

▲ Choose an appropriate MACS Column and 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 LS 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.
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells, representing the enriched satellite 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.
   ▲ **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-satellite cells by firmly pushing the plunger into the column.
6. (Optional) To further increase the purity of isolated satellite cells, e.g., for direct molecular analysis, the Anti-Integrin α-7 MicroBeads, mouse (# 130-104-261) can be used. For details refer to the respective data sheet.

Magnetic separation 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 non-satellite cells. Use the buffer composition as descibed in section 1.4 instead.

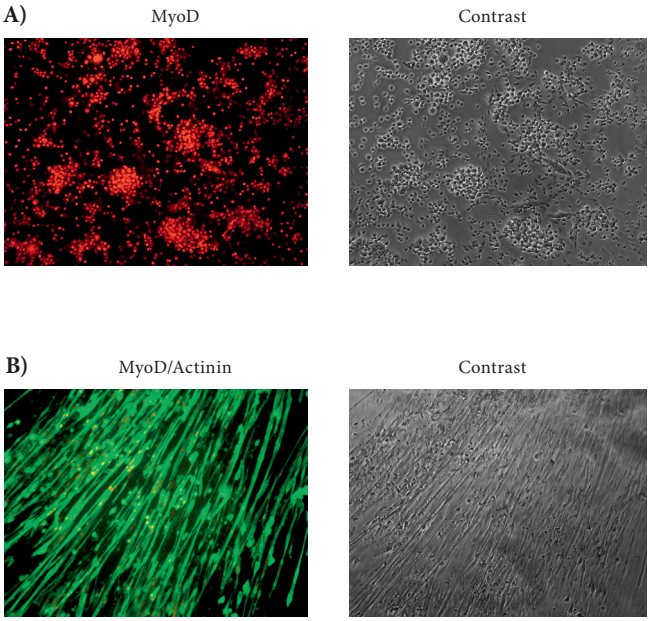
▲ Program choice depends on the isolation strategy, the strength of magnetic labeling, and the frequency of magnetically labeled non-satellite cells. For details refer to the section describing the cell separation programs in the respective user manual.

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. This fraction represents the enriched satellite cells.
4. (Optional) Collect positive fraction from row C. This fraction represents the magnetically labeled non-satellite cells.

3. Example of a separation using the Satellite Cell Isolation Kit

Mouse skeletal muscle was dissociated using the Skeletal Muscle Dissociation Kit, mouse and rat (# 130-098-305) in combination with the gentleMACS Dissociator. Subsequently, satellite cells were isolated using the Satellite Cell Isolation Kit, mouse and cultured in expansion medium (40% DMEM, 40% HAM's F10, 20% FBS, 2.5 ng/mL human FGF-2, 100 U/mL penicillin/ 100 U/mL streptomycin) for six days (A).
After seven days, medium was changed to differentiation medium (95% DMEM, 5% horse serum, 100 U/mL penicillin/ 100 U/mL streptomycin) and cells were incubated for three more days to induce differentiation and fusion into mature myotubes (B).



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

Warranty

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