Miltenyi Biotec

Anti-Integrin α-7 MicroBeads
mouse

Order no. 130-104-261

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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 Anti-Integrin α-7 MicroBeads, mouse: MicroBeads conjugated to monoclonal anti-mouse integrin α-7 antibodies.
Capacity	For 5×10^7 total cells, up to 50 separations.
Product format	Anti-Integrin α-7 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 integrin α-7$^+$ cells are magnetically labeled with Anti-Integrin α-7 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 integrin α-7$^+$ cells are retained within the column. The unlabeled cells run through. After removing the column from the magnetic field, the magnetically retained integrin α-7$^+$ cells can be eluted as the positively selected cell fraction.

1.2 Background information

Integrin α-7 (ITGA7) belongs to the integrin family of adhesion molecules. Integrins participate in important cell-cell and cell-extracellular matrix interactions in a diverse range of biological processes. ITGA7 is a heterodimer consisting of an α and a β subunit and is mainly expressed in skeletal and cardiac muscle. In skeletal muscle, ITGA7 is a marker for satellite cells[1]. It is a specific cellular receptor for the basement membrane protein laminin-1, as well as for the laminin isoforms-2 and -4 and is involved in differentiation and migration processes during myogenesis. Absence of ITGA7 results in muscular dystrophy. It is also required to promote contractile phenotype acquisition in differentiated airway smooth muscle cells.

1.3 Applications

- Enrichment of integrin α-7–expressing cells, e.g., satellite cells from mouse skeletal muscle.

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 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.

  ▲ **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.

  ▲ **Note:** Do not use ready-to-use buffers, e.g., autoMACS® Running Buffer or the MACSQuant® Running Buffer.

- MACS Columns and MACS Separators: Integrin α-7$^+$ cells can be enriched by using LS Columns. Positive selection can also be performed by using the autoMACS Pro Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Positive selection			
LS	10^8	2×10^9	MidiMACS, QuadroMACS, SuperMACS II
autoMACS	2×10^8	4×10^9	autoMACS Pro

  ▲ **Note:** Column adapters are required to insert certain columns into the 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) Fluorochrome-conjugated Anti-Integrin α-7 antibodies, mouse for flow cytometric analysis. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD Staining Solution (# 130-111-568) for flow cytometric exclusion of dead cells without fixation.
- (Optional) Dead Cell Removal Kit (# 130-090-101) for the depletion of dead cells.
- (Optional) Satellite Cell Isolation Kit, mouse (# 130-104-268) for depletion of non-satellite cells after tissue dissociation.
- (Optional) basic FGF/FGF2, e.g., Human FGF-2, premium grade (# 130-093-840) for culture of satellite cells.

2. Protocol

2.1 Sample preparation

When working with lymphoid organs, non-lymphoid tissues, or peripheral blood, prepare a single-cell suspension using manual methods or the gentleMACS Dissociator.

For details refer to the protocols section at www.miltenyibiotec.com/protocols.

▲ For isolation of satellite cells it is required to prepare a single-cell suspensions from murine skeletal muscle tissue using the Skeletal Muscle Dissociation Kit, mouse and rat (# 130-098-305) in combination with the gentleMACS Dissociator followed by depletion of non-satellite cells using the Satellite Cell Isolation Kit, mouse (# 130-104-268).

▲ Dead cells may bind non-specifically to MACS MicroBeads. To remove dead cells, we recommend using density gradient centrifugation or the Dead Cell Removal Kit (# 130-090-101).



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 10^6 total cells. When working with fewer than 10^6 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 2×10^6 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 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. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 80 µL of buffer per 10^6 cells.
4. Add 20 µL of Anti-Integrin α-7 MicroBeads per 10^6 total cells.
5. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
6. (Optional) Add staining antibodies according to manufacturer's recommendation.
7. Adjust volume to 500 µL using buffer for up to 5×10^6 cells.

   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
8. Proceed to magnetic separation (2.3).



2.3 Magnetic separation

▲ Choose an LS Column and MACS Separator according to the number of total cells and the number of integrin α-7$^+$ cells. 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.
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 as soon as the column reservoir is empty.
5. Remove column from the separator and place it on a suitable collection tube.
6. Pipette 5 mL of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.
7. (Optional) To increase the purity of integrin α-7$^+$ cells, the eluted fraction can be enriched over a second LS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

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Magnetic separation with the autoMACS® Pro Separator

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

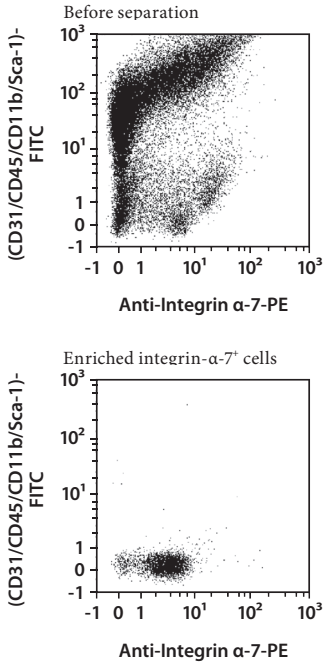
▲ Buffers used for operating the autoMACS Pro Separator should have a temperature of ≥10 °C.

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 one of the following programs:

   Positive selection: Posseld
   Collect positive fraction in row C of the tube rack.

3. Example of a separation using Anti-Integrin α-7 MicroBeads

Mouse skeletal muscle was dissociated using the Skeletal Muscle Dissociation Kit (# 130-098-305) in combination with the gentleMACS Dissociator. Non-satellite cells were depleted using the Satellite Cell Isolation Kit (# 130-104-268) followed by enrichment of satellite cells using Anti-Integrin α-7 MicroBeads. Cells were fluorescently stained with lineage markers (CD31/CD45/CD11b/Sca-1) in the FITC channel, Anti-Integrin α-7-PE, and CD34-APC 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.



4. References

1. Sacco, A. et al. (2008) Self-renewal and expansion of single transplanted muscle stem cells. Nature 456: 502–506.

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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