Miltenyi Biotec

CD61 MicroBeads
mouse and rat

Order no. 130-109-678

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 CD61 MicroBeads, mouse and rat: MicroBeads conjugated to monoclonal anti-rat CD61 antibodies (isotype: hamster IgG).
Capacity	For 5×10^8 total cells, up to 50 separations.
Product format	CD61 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 $CD61^+$ cells are magnetically labeled with CD61 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 $CD61^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of $CD61^+$ cells. After removing the column from the magnetic field, the magnetically retained $CD61^+$ cells can be eluted as the positively selected cell fraction.

1.2 Background information

CD61 MicroBeads, mouse and rat have been developed for the separation of cells expressing a 110 kDa integrin ß chain known as CD61 or Integrin ß3. Owing to multiple α/ß subunit combinations, integrins form an array of heterodimeric receptors, each recognizing a multitude of ligands. CD61, in association with either αv (CD51) or αIIb (CD41) integrin, binds to fibrinogen, fibronectin, von Willebrand factor, prothrombin, disintegrins and thrombospondin. Due to binding to various extracellular matrix proteins and cellular receptors, integrins play a significant role in cell adhesion and cell movement. Furthermore, a pro-apoptotic function of CD61 has been suggested using a hepatocellular carcinoma (HCC) cell line and patient tissues. Expression of CD61 is found on platelets, B cell subsets, myeloid cells, mast cells, endothelium, megakaryocytes, smooth muscle cells, and fibroblasts.

1.3 Applications

- Positive selection of cells expressing rat CD61 antigen.

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 rat serum albumin, rat 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: $CD61^+$ cells can be enriched by using LS Columns.

Column	Max. number of labeled cells	Max. number of total cells	Separator
LS	5×10^6	10^7	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.

- gentleMACS™ Dissociator (# 130-093-235), gentleMACS Octo Dissociator (# 130-095-937) or gentleMACS Octo Dissociator with Heaters (# 130-096-427) and gentleMACS C Tubes (# 130-093-237, # 130-096-334).
- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., Labeling Check Reagent-VioBlue® (# 130-095-087). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Neonatal Heart Dissociation Kit, mouse and rat (# 130-098-373) for the generation of single-cell suspension from neonatal rat heart.

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- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells.
- (Optional) MACS® SmartStrainers (70 µm) (# 130-098-462) to remove cell clumps after dissociation.
- (Optional) Pre-Separation Filters (70 µm) (# 130-095-823) to remove cell clumps before separation.
- (Optional) Dead Cell Removal Kit (# 130-090-101) for the depletion of dead cells.

2. Protocol

2.1 Sample preparation

For preparation of single-cell suspensions from neonatal rat heart use the Neonatal Heart Dissociation Kit, mouse and rat (# 130-098-373) with the gentleMACS™ Dissociator (# 130-093-235), the gentleMACS Octo Dissociator (# 130-095-937) or the gentleMACS Octo Dissociator with Heaters (# 130-096-427).

When working with lysed blood, prepare a single-cell suspension using standard methods.

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

When working with tissues, prepare a single-cell suspension using the gentleMACS Dissociator.

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

▲ 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 10^7 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 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 5 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 80 µL of buffer per 10^7 total cells.
4. Add 20 µL of CD61 MicroBeads per 10^7 total cells.
5. Mix well and incubate for 15 minutes in the dark in the refrigerator (2–8 °C).
6. (Optional) Add staining antibodies, e.g., 10 µL of Labeling Check Reagent-VioBlue® (# 130-95-087), and incubate for 5 minutes in the dark in the refrigerator (2–8 °C).
7. Adjust volume to 500 µL using buffer for up to 10^7 total cells. Do not centrifuge.
8. Proceed to magnetic separation (2.3).

2.3 Magnetic separation

▲ Choose an LS Column and an appropriate MACS 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 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 3×3 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.

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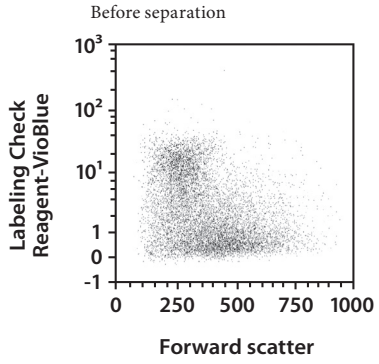
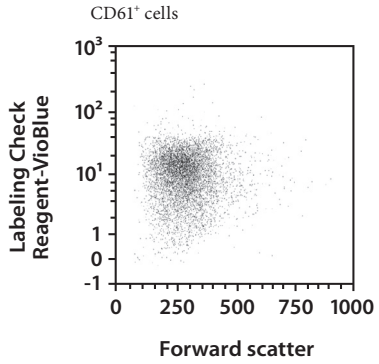
3. Example of a separation using CD61 MicroBeads

CD61$^+$ cells were isolated from a neonatal rat heart cell suspension using CD61 MicroBeads, an LS Column, and a MidiMACS™ Separator. Cells were fluorescently stained with Labeling Check Reagent-VioBlue® 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.





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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