Miltenyi Biotec

Tissue Stem Cell Pre-Enrichment Kit
mouse

Order no. 130-109-205

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 Tissue Stem Cell Pre-Enrichment Cocktail, mouse:** MicroBeads conjugated to monoclonal anti-mouse antibodies.
Capacity	For 5×10^8 total cells, 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

First, the committed and differentiated cells are magnetically labeled with a cocktail of monoclonal antibodies conjugated with MACS® 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 cells are retained within the column. The unlabeled tissue stem cells run through. After removing the column from the magnetic field, the magnetically retained lineage cells can be eluted.

1.2 Background information

The Tissue Stem Cell Pre-Enrichment Kit, mouse has been designed for the pre-enrichment of untouched tissue resident cell populations, e.g., stem and progenitor cells, from primary specimens. All primary tissues are vascularized and infiltrated by heterogeneous lymphocyte subpopulations, red blood cells, and endothelial cells. The level of heterogeneity is highly dependent on multiple factors like tissue type, age, and disease state.
The isolation of target cells from primary tissues, such as organ specific stem and progenitor cells, is frequently hampered by the initial low frequency of these cells. This may lead to long isolation times and impaired purities. By using the Tissue Stem Cell Pre-Enrichment Kit, mouse all generic infiltrating cells, such as red and white blood cells as well as endothelial cells are efficiently depleted resulting in a significant pre-enrichment of target cells for downstream analysis or sorting.

1.3 Applications

- Enrichment of untouched tissue–resident stem and progenitor cells.

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.
  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
LS	4×10^7	5×10^7	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
autoMACS	5×10^7	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) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., Anti-Ter-119-APC, mouse (# 130-102-290), CD31-APC, mouse (# 130-102-571), CD45-APC, mouse

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macsde@miltenyi.com
www.miltenyibiotec.com

140-004-972.02

(# 130-102-544), Labeling Check Reagent-VioBlue® (# 130-095-087). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.

- gentleMACS™ Dissociator (# 130-093-235), gentleMACS Octo Dissociator (# 130-095-937), or gentleMACS Octo Dissociator with Heaters (# 130-096-427)
- gentleMACS C Tubes (# 130-093-237, # 130-096-334)
- Pre-Separation Filters, 70 µm (# 130-095-823) to remove cell clumps.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells.

2. Protocol

2.1 Sample preparation

For preparation of a single-cell suspension from solid tissues it is recommended to use gentleMACS Dissociators in combination with tissue-specific dissociation kits.

▲ 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 cells from dissociated tissue. When working with less cells, use the same volumes as indicated. When working with higher amounts, scale up all reagent volumes and total volumes accordingly to the final cell number of dissociated tissue (e.g. for 2×10^7 cells, use twice the volume of all indicated reagent volumes and total volumes). It is strongly recommended to determine the actual cell number from dissociated tissue prior to magnetic labeling.

▲ 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 Tissue Stem Cell Pre-Enrichment Cocktail per 10^7 total cells.
5. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
6. Adjust volume to 500 µL with buffer for up to 5×10^7 total cells.
   ▲ **Note:** If more tissue was used split sample into multiple tubes.
7. Proceed to magnetic separation (2.3).

2.3 Magnetic separation: Depletion of lineage-positive cells

▲ Choose an LS Column and MACS Separator. For details refer to 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 stem and progenitor 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. (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.

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:

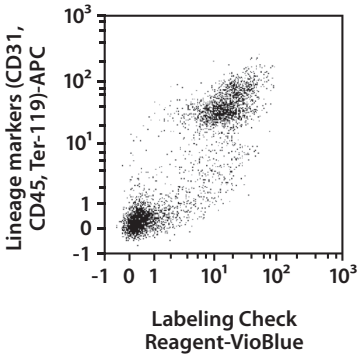
   Depletion: Depletes
   Collect negative fraction in row B of the tube rack. This fraction represents the enriched stem and progenitor cells.
4. (Optional) Collect positive fraction from row C of the tube rack. This fraction represents the magnetically labeled non–target cells.

140-004-972.02

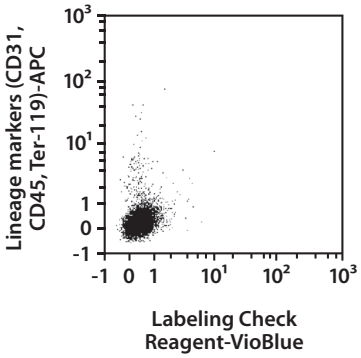
3. Example of a separation using the Tissue Stem Cell Pre-Enrichment Kit

Mouse mammary gland was dissociated using the Adipose Tissue Dissociation Kit, mouse and rat (# 130-105-808) in combination with the gentleMACS Octo Dissociator. Subsequently, tissue–resident stem and progenitor cells were pre-enriched using the Tissue Stem Cell Pre-Enrichment Kit, mouse. Cells were fluorescently stained with lineage markers (CD31, CD45, Ter-119 conjugated to APC) and 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.

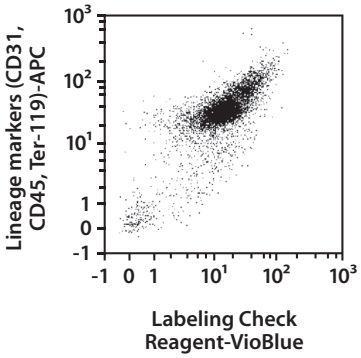
Before separation



Stem cell-enriched fraction



Lineage cell-enriched fraction



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.

Legal notices

Limited product warranty

Miltenyi Biotec B.V. & Co. KG and/or its affiliate(s) warrant this product to be free from material defects in workmanship and materials and to conform substantially with Miltenyi Biotec's published specifications for the product at the time of order, under normal use and conditions in accordance with its applicable documentation, for a period beginning on the date of delivery of the product by Miltenyi Biotec or its authorized distributor and ending on the expiration date of the product's applicable shelf life stated on the product label, packaging or documentation (as applicable) or, in the absence thereof, ONE (1) YEAR from date of delivery ("Product Warranty"). Miltenyi Biotec's Product Warranty is provided subject to the warranty terms as set forth in Miltenyi Biotec's General Terms and Conditions for the Sale of Products and Services available on Miltenyi Biotec's website at www.miltenyibiotec.com, as in effect at the time of order ("Product Warranty"). Additional terms may apply. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING IF A PRODUCT IS SUITABLE FOR CUSTOMER'S PARTICULAR PURPOSE AND APPLICATION METHODS.

Technical information

The technical information, data, protocols, and other statements provided by Miltenyi Biotec in this document are based on information, tests, or experience which Miltenyi Biotec believes to be reliable, but the accuracy or completeness of such information is not guaranteed. Such technical information and data are intended for persons with knowledge and technical skills sufficient to assess and apply their own informed judgment to the information. Miltenyi Biotec shall not be liable for any technical or editorial errors or omissions contained herein.

All information and specifications are subject to change without prior notice. Please contact Miltenyi Biotec Technical Support or visit www.miltenyibiotec.com for the most up-to-date information on Miltenyi Biotec products.

Licenses

This product and/or its use may be covered by one or more pending or issued patents and/or may have certain limitations. Certain uses may be excluded by separate terms and conditions. Please contact your local Miltenyi Biotec representative or visit Miltenyi Biotec's website at www.miltenyibiotec.com for more information.

The purchase of this product conveys to the customer the non-transferable right to use the purchased amount of the product in research conducted by the customer (whether the customer is an academic or for-profit entity). This product may not be further sold. Additional terms and conditions (including the terms of a Limited Use Label License) may apply.

CUSTOMER'S USE OF THIS PRODUCT MAY REQUIRE ADDITIONAL LICENSES DEPENDING ON THE SPECIFIC APPLICATION. THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING FOR ITSELF WHETHER IT HAS ALL APPROPRIATE LICENSES IN PLACE. Miltenyi Biotec provides no warranty that customer's use of this product does not and will not infringe intellectual property rights owned by a third party. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

Trademarks

autoMACS, gentleMACS, MACS, MACSQuant, MidiMACS, the Miltenyi Biotec logo, QuadroMACS, SuperMACS, VarioMACS, and VioBlue are registered trademarks or trademarks of Miltenyi Biotec and/or its affiliates in various countries worldwide.