Miltenyi Biotec

Lineage Cell Depletion Kit
human

Order no. 130-092-211

Index

1. Description

Components

1 mL Biotin-Antibody Cocktail:
Cocktail of biotin-conjugated monoclonal antibodies against CD2, CD3, CD11b, CD14, CD15, CD16, CD19, CD56, CD123, and CD235a (Glycophorin A).

2 mL Anti-Biotin MicroBeads:
MicroBeads conjugated to a monoclonal anti-biotin antibody (isotype: mouse IgG1).

Size
For 10^9 total cells, up to 100 separations.

Product format
The Biotin-Antibody Cocktail is supplied in a solution containing stabilizer and 0.05% sodium azide. The Anti-Biotin MicroBeads are supplied as a suspension containing stabilizer and 0.05% sodium azide.

Storage
Store protected from light at 4–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Principle of MACS® separation

Using the Lineage Cell Depletion Kit, lineage negative cells are isolated by depletion of cells expressing so-called "lineage" antigens (untouched isolation).
Lineage$^+$ cells are indirectly magnetically labeled with a cocktail of biotin-conjugated monoclonal antibodies as primary labeling reagent and anti-biotin monoclonal antibodies conjugated to MicroBeads as secondary labeling reagent. The magnetically labeled lineage cells are depleted by retaining them on a MACS® Column in the magnetic field of a MACS Separator while the unlabeled lineage negative cells pass through the column.

1.2 Background and product applications

The Lineage Cell Depletion Kit is an indirect magnetic labeling system for the depletion of mature hematopoietic cells such as T cells, B cells, NK cells, dendritic cells, monocytes, granulocytes, erythroid cells, and their committed precursors from mononuclear cells from bone marrow, cord blood, or mobilized leukapheresis products. The depletion of lineage$^+$ cells results in the enrichment of untouched stem and progenitor cells.

For depletion, cells are magnetically labeled by using a cocktail of biotin-conjugated antibodies against a panel of so-called "lineage" antigens: CD2, CD3, CD11b, CD14, CD15, CD16, CD19, CD56, CD123, and CD235a (Glycophorin A), and Anti-Biotin MicroBeads.

Examples of applications

- Enrichment of lineage-negative cells from human bone marrow, cord blood, and leukapheresis.
- Enrichment of hematopoietic *in vitro* colony-forming cells (CFC, CFU-GM, BFU-E and CFU-Mk) and primitive long-term culture-initiating cells.
- Enrichment of nonhematopoietic *in vitro* colony-forming cells (CFU-Fs) from bone marrow.

1.3 Reagent and instrument requirements

- Buffer (degassed): Prepare a solution containing PBS (phosphate buffered saline) pH 7.2, 0.5% 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 (4–8 °C).

  ▲ **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 human serum albumin, human serum or fetal calf serum. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- MACS Columns and MACS Separators: Choose the appropriate MACS Separator and MACS Columns according to the number of labeled cells and to the number of total cells.

Column	max. number of labeled cells	max. number of total cells	Separator
MS	10^7	2×10^8	MiniMACS, OctoMACS, VarioMACS, SuperMACS
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
XS	10^9	2×10^{10}	SuperMACS
autoMACS	2×10^8	4×10^9	autoMACS

▲ **Note:** Column adapters are required to insert certain columns into VarioMACS™ Separator or SuperMACS™ Separator. For details, see MACS Separator data sheets.

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

140-001-644.05

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g. CD34-FITC (# 130-081-001); CD34-PE (# 130-081-002); CD34-APC (# 130-090-954), CD133/2 (293C3)-PE (# 130-090-853), CD133/2 (293C3)-APC (# 130-090-854), CD271 (LNGFR)-PE (# 130-091-885), CD271 (LNGFR)-APC (# 130-091-884); Anti-Biotin-PE (# 130-090-756), Anti-Biotin-APC (# 130-090-856).
- (Optional) Pre-Separation Filters (# 130-041-407) to remove cell clumps.
- (Optional) PI (propidium iodide) or 7-AAD for the flow cytometric exclusion of dead cells.
- (Optional) MACS HSC-CFU media for the evaluation of the hematopoietic engraftment potential of the lineage-depleted cell fraction. For product detail, see catalog or website.
- (Optional) MACS NH CFU-F Medium for the evaluation and quantification of nonhematopoietic stem cells in the lineage-depleted cell fraction. For product detail, see catalog or website.

2. Protocol

2.1 Sample preparation

When working with anticoagulated bone marrow or cord blood, mononuclear cells should be isolated by density gradient centrifugation (e.g. Ficoll-Paque™, see "General protocols" in the User Manuals or visit www.miltenyibiotec.com/protocols).

▲ **Note:** Remove platelets after density gradient separation: resuspend cell pellet in buffer and centrifuge at 200×g for 10–15 minutes at 20 °C. Carefully remove supernatant. Repeat washing step and carefully remove supernatant.

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 separation. Pass cells through 30 µm nylon mesh (Pre-Separation Filters # 130-041-407) to remove cell clumps which may clog the column.

▲ Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Pipette off supernatant completely.
3. Resuspend cell pellet in 40 µL of buffer per 10^7 total cells.
4. Add 10 µL of Biotin-Antibody Cocktail per 10^7 total cells.
5. Mix well and incubate for 10 minutes at 4–8 °C.
6. Wash cells carefully with buffer by adding 500–1000 µL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Pipette off supernatant completely.
7. Resuspend cell pellet carefully in 80 µL of buffer per 10^7 total cells.
8. Add 20 µL of Anti-Biotin MicroBeads per 10^7 total cells.
9. Mix well and incubate for an additional 15 minutes at 4–8 °C.
10. Wash cells carefully with buffer by adding 500–1000 µL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Pipette off supernatant completely.
11. Resuspend up to 10^8 cells in 500 µL of buffer.

    ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
12. Proceed to magnetic separation.

2.3 Magnetic separation

▲ Choose an appropriate MACS Column and MACS Separator according to the number of labeled cells and to the number of total cells (see table in section 1.3).

▲ **Note:** When working with bone marrow cells, the number of labeled cells is almost equal to the number of total cells. Be careful and do not exceed the column capacity for labeled cells.

Magnetic separation with MS and LS Columns

1. Place column in the magnetic field of a suitable MACS Separator (see "Column data sheets").
2. Prepare column by rinsing with appropriate amount of buffer:
   MS: 500 µL LS: 3 mL
3. Apply cell suspension onto the column.
4. Allow the cells to pass through and collect effluent, as fraction with unlabeled cells represent the enriched lineage negative cells.
5. Wash column with appropriate amount of buffer. Perform washing steps by adding buffer three times, each time once the column reservoir is empty.
   MS: 3×500 µL LS: 3×3 mL

   Collect entire effluent in the same tube as effluent of step 4. This fraction represents the enriched lineage negative cells.
6. (Optional) Elute retained cells outside of the magnetic field. This fraction represents the magnetically labeled lineage$^+$ cells.

Magnetic separation with XS Columns

For instructions on the column assembly and the separation, refer to the "XS Column data sheet".

Magnetic separation with the autoMACS™ Separator

▲ Refer to the "autoMACS™ User Manual" for instructions on how to use the autoMACS Separator.

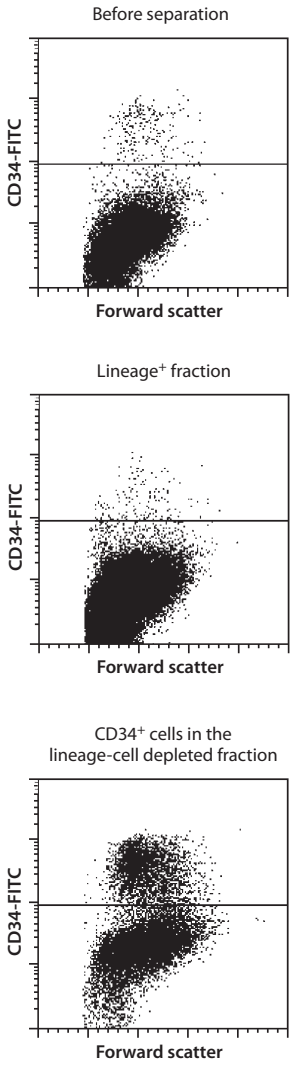
1. Prepare and prime autoMACS Separator.
2. Place tube containing the magnetically labeled cells in the autoMACS Separator. Choose program "Deplete".
3. Collect negative fraction (outlet port "neg1"). This fraction represents the enriched lineage negative cells.
4. (Optional) Collect positive fraction (outlet port "pos1"). This fraction represents the magnetically labeled lineage$^+$ cells.

2.4 (Optional) Evaluation of lineage negative cell purity

The purity of the enriched lineage-negative cells can be evaluated by flow cytometry or fluorescence microscopy. Stain aliquots of the cell fractions with a fluorochrome-conjugated antibody against CD133, e.g. CD133/2 (293C3)-APC (# 130-090-854) or CD133/2 (293C3)-PE (# 130-090-853), and optional against CD34, e.g. CD34-FITC (# 130-081-001), for the staining of hematopoietic progenitor cells. CD271 (LNGFR)-FITC (# 130-091-917) can be used for staining of nonhematopoietic (NH) stem and progenitor cells contained in lineage-negative fractions of bone marrow. The staining procedure should be performed as recommended in the respective data sheets. Analyze cells by flow cytometry or fluorescence microscopy. Labeling of lineage$^+$ cells with the Biotin-Antibody Cocktail can be visualized by counterstaining with a fluorochrome-conjugated anti-biotin antibody, e.g. Anti-Biotin-PE (# 130-090-756) or Anti-Biotin-APC (# 130-090-856). Staining with fluorochrome-conjugated streptavidin is not recommended.

3. Example of a separation using the Lineage Cell Depletion Kit

Isolation of untouched lineage-negative cells from bone marrow using the Lineage Cell Depletion Kit and an LS Column. Cells are fluorescently stained with CD34-FITC (# 130-081-001). Cell debris and dead cells were excluded from the analysis based on scatter signals and PI 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.

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.

140-001-644.05

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, MACS, MidiMACS, the Miltenyi Biotec logo, MiniMACS, OctoMACS, QuadroMACS, SuperMACS, and VarioMACS are registered trademarks or trademarks of Miltenyi Biotec and/or its affiliates in various countries worldwide. All other trademarks mentioned in this publication are the property of their respective owners and are used for identification purposes only.

Ficoll-Paque is a trademark of GE Healthcare companies.