Miltenyi Biotec

CD105 MultiSort Kit (PE)
mouse

Order no. 130-092-924

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1. Description

Components

2 mL CD105-PE, mouse:
Monoclonal CD105 antibody conjugated to R-phycoerythrin (PE) (isotype: rat IgG2a).

2 mL Anti-PE MultiSort MicroBeads:
MultiSort MicroBeads conjugated to monoclonal anti-PE Isomer-1 antibody (isotype: mouse IgG1; clone PE4-14D10).

2×1 mL MultiSort Release Reagent

3 mL MultiSort Stop Reagent

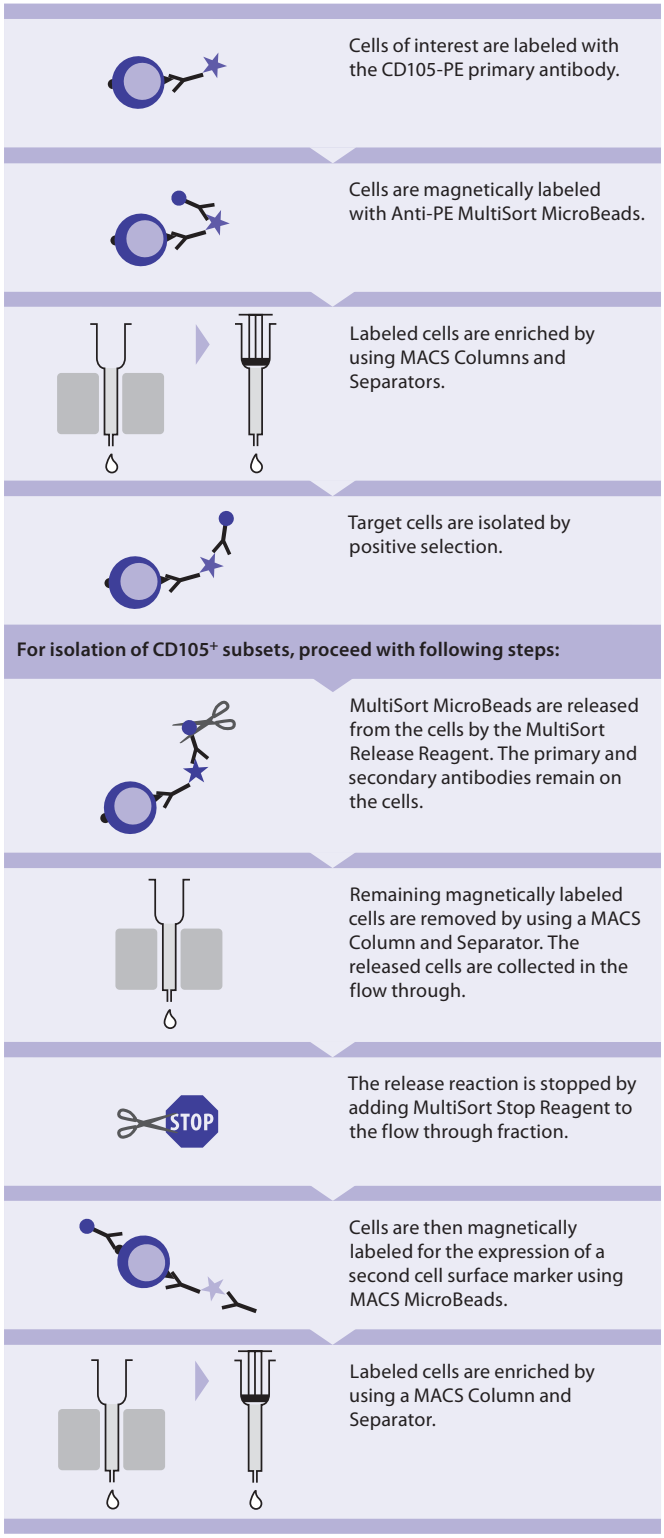
Size
For 10^9 total cells.

Product format
CD105-PE, Anti-PE MultiSort MicroBeads, and the MultiSort Release Reagent are supplied in buffer containing stabilizer and 0.05% sodium azide.

The MultiSort Stop Reagent is supplied in buffer containing 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



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

1.2 Background information

The CD105 MultiSort Kit (PE) was developed to enable the magnetic isolation of subpopulations of CD105$^+$ cells, for example, CD105$^+$ Sca-1$^+$ hematopoietic stem cells. CD105, also known as endoglin, is a proliferation-associated and hypoxia-inducible protein abundantly expressed in angiogenic endothelial cells.[1] In mouse bone marrow (BM), CD105 was also found to be expressed on a population of Sca-1$^+$ hematopoietic stem cells (HSCs), which were further characterized to exclusively possess a long-term repopulating (LTR) ability in mice, and are therefore termed LTR-HSCs.[2,3]
The selection of CD105$^+$ cells is therefore important not only in certain areas of stem cell research, but also in the study of angiogenic endothelial cells, particularly in the development and maintenance of solid tumors[1]. In addition, CD105 has been used for the isolation of murine cardiac endothelial cells.[4]

1.3 Applications

- Isolation of CD105$^+$Sca-1$^+$ LTR-HSCs from mouse bone marrow cells in conjunction with Anti-Sca-1 MicroBead Kit (FITC) (# 130-092-529). For details see special protocol "Isolation of CD105$^+$Sca-1$^+$ cells from mouse bone marrow", available at www.miltenyibiotec.com/protocols.
- Isolation of endothelial cells, for example, from murine cardiac tissue.

  ▲ **Note:** Please refer to section 2.5 for a generic protocol when using cell sources other than mouse bone marrow 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.

  ▲ **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 bovine serum. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- MACS Columns and MACS Separators: Cells can be enriched (positive selection) by using MS or LS Columns. Positive selection can also be performed by using the autoMACS™ or the autoMACS Pro Separator.

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
autoMACS	2×10^8	4×10^9	autoMACS, autoMACS Pro

  ▲ **Note:** Column adapters are required to insert certain MS or LS Columns into the VarioMACS™ or SuperMACS™ Separators. For details see the respective MACS Separator data sheet.

- (Optional) Fluorochrome-conjugated antibody for flow cytometric analysis, e.g., CD117-APC (# 130-091-729) or the Lineage Cell Detection Cocktail-Biotin, mouse (# 130-092-613). Due to autofluorescence of endothelial cells (PE) the use of CD105 antibodies conjugated to APC are recommended. For information about fluorochrome-conjugates see www.miltenyibiotec.com.
- (Optional) Propidium iodide (PI) or 7-AAD for flow cytometric exclusion of dead cells.
- (Optional) Dead Cell Removal Kit (# 130-090-101) for the depletion of dead cells.
- (Optional) Pre-Separation Filters (# 130-041-407) to remove cell clumps.
- (Optional) Anti-Sca-1 MicroBead Kit (FITC) (# 130-092-529) for the selection of CD105$^+$ Sca-1$^+$ LTR-HSCs. For details see special protocol "Isolation of CD105$^+$Sca-1$^+$ cells from mouse bone marrow", available at www.miltenyibiotec.com/protocols.

2. Protocol

2.1 Preparation of bone marrow cells

For preparation of murine bone marrow cells please refer to the protocol "Preparation of single-cell suspensions from mouse bone marrow" at www.miltenyibiotec.com/protocols.



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. Wet filter with buffer before use.

▲ The concentration of Anti-PE MultiSort MicroBeads used to achieve an optimal magnetic separation and release of MultiSort MicroBeads is primarily dependent on the intensity of CD105-PE antibody staining and, to some degree, also on the frequency of target cells in suspension. Dimly PE-stained target cells require a higher concentration of Anti-PE MultiSort MicroBeads to achieve optimal magnetic labeling and separation. Target cells with a high frequency (>50%) may also require a higher concentration of Anti-PE MultiSort MicroBeads than target cells with lower frequencies. For details see table below.

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

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 10^7 total cells.
4. Add 20 µL of CD105-PE per 10^7 total cells.

   ▲ **Note:** If the second parameter sorting is to be performed using indirect MicroBeads, we recommend to simultaneously label cells with the CD105-PE primary antibody as well as the primary antibody conjugate to be used in the second parameter. Reduce the volume of the buffer accordingly to accomodate both antibodies in their optimal staining concentration. For the primary antibody conjugate to be used in the second parameter use a staining concentration according to manufacturer´s recommendations.

5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C) or according to the manufacturer's recommendations.
6. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. (Optional) Repeat washing step.
8. Resuspend cell pellet in 80 µL of buffer and add 20 µL of Anti-PE MultiSort MicroBeads according to recommendations in the table below. For more details see also section 2.5.

Buffer and MicroBead volumes per 10^7 total cells

Antigen staining	Buffer	Anti-PE MultiSort MicroBeads
"dim"	80 µL	20 µL
"intermediate"	**90 µL**	**10 µL**
"bright"	96 µL	4 µL

▲ **Nwe:** A too strong dilution of the Anti-PE MultiSort MicroBeads may result in poor retention of the PE-labeled target cells. A too low dilution may result in less efficient release of MicroBeads.

9. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
10. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate 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).



2.3 Magnetic separation

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of magnetically labeled cells. For details see table in section 1.4.

Magnetic separation with MS or LS Columns

▲ To achieve highest purities, perform two consecutive column runs.

1. Place column in the magnetic field of a suitable MACS Separator. For details see the respective MACS Column data sheet.
2. Prepare column by rinsing with the appropriate amount of buffer:
   MS: 500 µL LS: 3 mL
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with appropriate amount of buffer. Collect total effluent; this is the unlabeled cell fraction. Perform washing steps by adding buffer three times. Only add new buffer when the column reservoir is empty.
   MS: 3×500 µL LS: 3×3 mL
5. Remove column from the separator and place it on a suitable collection tube.
   ▲ **Note:** To perform a second column run, you may elute the cells directly from the first onto the second, equilibrated column instead of a collection tube.
6. Pipette the appropriate amount of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.
   MS: 1 mL LS: 5 mL
7. To increase purity of $CD105^+$ cells, the eluted fraction can be enriched over a second MS or LS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.
8. Proceed to removal of MultiSort MicroBeads (2.4).

Magnetic separation with the autoMACS® Separator or the autoMACS® Pro Separator

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

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

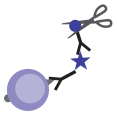
▲ Program choice depends on the isolation strategy, the strength of magnetic labeling, and the frequency of magnetically labeled cells. For details refer to the section describing the cell separation programs in the respective user manual.

Magnetic separation with the autoMACS® Separator

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 at the uptake port and the fraction collection tubes at port neg1 and port pos2.
3. For a standard separation choose one of the following programs:
   Positive selection: posseld2
   Collect positive fraction from outlet port pos2.
4. Proceed to removal of MultiSort MicroBeads (2.4).

Magnetic separation with the autoMACS® Pro Separator

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 fraction collection tubes in rows B and C.
3. For a standard separation choose one of the following programs:
   Positive selection: posseld2
   Collect positive fraction in row C of the tube rack.
4. Proceed to removal of MultiSort MicroBeads (2.4).



2.4 (Optional) Removal of MultiSort MicroBeads and second magnetic labeling and separation

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

1. Remove a sample for analysis by flow cytometry and proceed with the remaining magnetically labeled fraction.
2. Add 20 µL of MultiSort Release Reagent per 1 mL of cell suspension.

3. Mix well and incubate for 10 minutes in the refrigerator in the dark (2–8 °C).
4. To remove any residual magnetically labeled cells, repeat the magnetic separation procedure as described in 2.3. Separate cells over a new column of the same type (MS or LS Column) or use the autoMACS or autoMACS Pro program "possel". For details see section 2.5.
5. Wash cells from the released fraction carefully by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
6. Resuspend cells in buffer at a final concentration of 10^7 total cells per 50 µL of buffer.
7. Add 30 µL of MultiSort Stop Reagent per 10^7 total cells and mix well.
8. Add the recommended amount of direct or indirect MACS MicroBeads (see the respective MACS MicroBead data sheet) to magnetically label the cells for the second marker. Adjust to 100 µL total volume by adding buffer.

   ▲ **Note:** The Anti-CD105 antibody is of a rat IgG2a isotype with kappa light chains. Thus, Goat Anti-Rat IgG MicroBeads or Mouse Anti-Rat Kappa MicroBeads cannot be used for second parameter sorting. When using other Anti-Immunoglobulin MicroBeads for the second parameter sorting, any reactivity with the isotype of the primary antibody of the first parameter sorting must be avoided.
9. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
10. Proceed to magnetic separation (2.3). For details see the respective MACS MicroBeads data sheet.

2.5 Recommendations for the optimization of cell isolation using the CD105 MultiSort Kit (PE) for other cell sources than mouse bone marrow.

How efficient the MultiSort MicroBeads are released from cell surface is dependent upon the strength of the magnetic labeling. This, in turn, is dependent upon the intensity of staining with the primary CD105-PE antibody and on the amount of Anti-PE MultiSort MicroBeads used for magnetic labeling. For details see table in section 2.2. Typically, a release of >90% is obtained when working with the Anti-PE MultiSort MicroBeads.

$$\textbf{Release (\%)}: \frac{100 \times (\text{No. of cells in released fraction})}{(\text{No. of cells in released + unreleased fractions})}$$

Too strong magnetic labeling due to a too high concentration of the Anti-PE MultiSort MicroBeads results in an insufficient release of the Anti-PE MultiSort MicroBeads. An insufficient release decreases the purity of the cells after positive selection according to the second parameter.

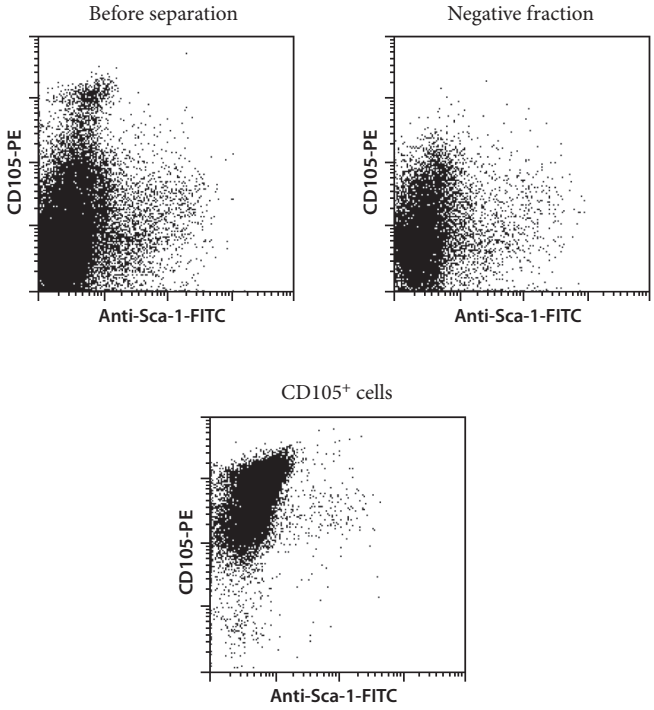
▲ Use a lower amount of Anti-PE MultiSort MicroBeads if the recovery of the target cells after the primary parameter sorting with the Anti-PE MultiSort MicroBeads is sufficient but the release is <90%.

Too weak magnetic labeling due to a too low concentration of the Anti-PE MultiSort MicroBeads results in low recovery of the PE-labeled target cells in the positive fraction.

▲ Use more Anti-PE MultiSort MicroBeads if the release is efficient (>90%) but the recovery of the positive cells after the first positive selection with the Anti-PE MultiSort MicroBeads is too low.

3. Example of a separation using the CD105 MultiSort Kit (PE)

CD105+ cells were separated from mouse bone marrow using the CD105 MultiSort Kit (PE), MS Columns, and a MiniMACS™ Separator. Cells are fluorescently stained with CD105-PE and Anti-Sca-1-FITC (# 130-093-222). Cell debris and dead cells are excluded from the analysis based on scatter signals and PI fluorescence.



4. References

1. Duff, S. E. *et al.* (2003) CD105 is important for angiogenesis: evidence and potential applications. FASEB J. 17: 984–992.
2. Chen, C. Z. *et al.* (2002) Identification of endoglin as a functional marker that defines long-term repopulating hematopoietic stem cells. Proc. Natl. Acad. Sci. USA 99: 15468–15473.
3. Chen, C. Z. *et al.* (2003) The endoglin^{positive} Sca-1^{positive} rhodamine^{low} phenotype defines a hear-homogeneous population of long-term repopulating stem cells. Immunity 19: 525–533.
4. Huang, M. T. *et al.* (2005) Endothelial intercellular adhesion molecule (ICAM)-2 regulates angiogenesis. Blood 106: 1636–1643.

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.

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