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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	2 mL CD90 MicroBeads, human: MicroBeads conjugated to monoclonal anti-human CD90 antibodies (mouse IgG1).
Capacity	For 10 ⁹ total cells, up to 100 separations.
Product format	CD90 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 CD90⁺ cells are magnetically labeled with CD90 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 CD90⁺ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of CD90⁺ cells. After removing the column from the magnetic field, the magnetically retained CD90⁺ cells can be eluted as the positively selected cell fraction. To increase the purity, the positively selected cell fraction containing the CD90⁺ cells must be separated over a second column.

1.2 Background information

The CD90 antibody reacts with the human CD90 antigen, also known as Thy-1, a 25–35 kD GPI-anchored protein of the Ig superfamily involved in cell-cell and cell-matrix interactions. CD90 was identified as a marker for cancer stem cells in human liver cancer.¹ In addition to cancer stem cells, it is expressed on embryonic stem cells (ESC), induced pluripotent stem cells (iPSC), neurons, small subsets of human fetal liver cells and thymocytes, cord blood, and bone marrow cells. CD90 is also expressed on a subset of CD34⁺ primitive hematopoietic stem cells. CD90⁺CD34⁺ cells characterize a highly enriched population of high proliferative potential colony-forming cells (HPP-CFC).² Furthermore, CD90 expression is found on mesenchymal stem cells (MSCs).³

1.3 Applications

- Positive selection or depletion of cells expressing human CD90 antigen, e.g., liver cancer stem 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). 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 human serum albumin, human serum, or fetal bovine serum (FBS). Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.

- MACS Columns and MACS Separators: CD90⁺ cells can be enriched by using MS or LS Columns or depleted with the use of LD Columns. Cells that strongly express the CD90 antigen can also be depleted using MS or LS Columns. Positive selection or depletion can also be performed by using the autoMACS Pro Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Positive selection			
MS	10 ⁷	2×10 ⁷	MiniMACS, OctoMACS, VarioMACS, SuperMACS II
LS	2×10 ⁷	4×10 ⁷	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
Depletion			
LD	1.5×10 ⁷	3×10 ⁷	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
Positive selection or depletion			
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 CD90 antibodies for flow cytometric analysis, e.g., CD90-PE and CD326 (EpCAM)-APC. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) 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 (30 μm) (# 130-041-407) to remove cell clumps.

2. Protocol

2.1 Sample preparation

When working with anticoagulated peripheral blood or buffy coat, peripheral blood mononuclear cells (PBMCs) should be isolated by density gradient centrifugation, for example, using Ficoll-Paque™.

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

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

▲ Cells can be labeled with MACS MicroBeads using the autolabeling function of the autoMACS Pro Separator. For more information refer to section 2.4.

▲ 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 10⁷ total cells. When working with fewer than 10⁷ 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 2×10⁷ 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 30 μm nylon mesh (Pre-Separation Filters (30 μm) # 130-041-407) 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 10⁷ total cells.
4. Add 20 μL of CD90 MicroBeads per 10⁷ 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 CD90-PE, and incubate for 5 minutes in the dark in the refrigerator (2–8 °C).
7. Wash cells by adding 1–2 mL of buffer per 10⁷ cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
8. Resuspend up to 10⁸ cells in 500 μL of buffer.
 - ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
 - ▲ **Note:** For depletion with LD Columns, resuspend up to 1.25×10⁸ cells in 500 μL of buffer.
9. 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 CD90⁺ 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 MS or 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 the appropriate amount of buffer:

MS: 500 µL LS: 3 mL

3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.

4. Wash column with the appropriate amount of buffer. Collect unlabeled cells that pass through and combine with the flow-through from step 3.

MS: 3×500 µL LS: 3×3 mL

▲ **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 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. (Optional) To increase the purity of CD90⁺ 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.

Depletion with LD Columns

1. Place LD Column in the magnetic field of a suitable MACS Separator. For details refer to the LD Column data sheet.

2. Prepare column by rinsing with 2 mL of buffer.

3. Apply cell suspension onto the column.

4. Collect unlabeled cells that pass through and wash column with 2×1 mL of buffer. Collect total flow-through; this is the unlabeled cell fraction. Perform washing steps by adding buffer two times. Only add new buffer when the column reservoir is empty.

2.4 Cell separation with the autoMACS[®] Pro Separator

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

▲ All buffer temperatures should be ≥10 °C.

▲ For appropriate resuspension volumes and cell concentrations, please visit www.automacspro.com/autolabeling.

▲ Place tubes in the following Chill Rack positions: position A = sample, position B = negative fraction, position C = positive fraction.

2.4.1 Fully automated cell labeling and separation

1. Switch on the instrument for automatic initialization.

2. Go to the **Reagent** menu and select **Read Reagent**. Scan the 2D barcode of each reagent vial with the barcode scanner on the autoMACS Pro Separator. Place the reagent into the appropriate position on the reagent rack.

3. Place sample and collection tubes into the Chill Rack.

4. Go to the **Separation** menu and select the reagent name for each sample from the **Labeling** submenu (the correct labeling, separation, and wash protocols will be selected automatically).

5. Enter sample volume into the **Volume** submenu. Press **Enter**.

6. Select **Run**.

2.4.2 Magnetic separation with the autoMACS[®] Pro Separator using manual labeling

1. Label the sample as described in section 2.2 Magnetic labeling.

2. Prepare and prime the instrument.

3. 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.

4. For a standard separation choose the following program:

Positive selection: Posseld2

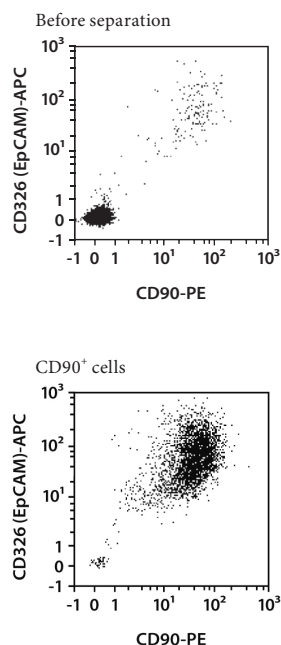
Collect positive fraction in row C of the tube rack.

Depletion: Deplete

Collect negative fraction in row B of the tube rack.

3. Example of a separation using CD90 MicroBeads

CD90⁺ human teratocarcinoma cells (NTERA2) were isolated from a mixture of U937 and NTERA2 cells using CD90 MicroBeads, an MS Column, and an OctoMACS™ Separator. Cells were fluorescently stained with CD90-PE and CD326 (EpCAM)-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. Zhen Fan Yang, Z. F. *et al.* (2008) Significance of CD90⁺ Cancer Stem Cells in Human Liver Cancer. *Cancer Cell* 13: 153–166.
2. Mayani, H. and Lansdorp, P. M. *et al.* (1994) Thy-1 expression is linked to functional properties of primitive hematopoietic progenitor cells from human umbilical cord blood. *Blood* 83: 2410–2417.
3. Dominici, M. *et al.* (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytherapy* 8: 315–317.

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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