Miltenyi Biotec

CD133 MicroBead Kit – Tumor Tissue
human

Order no. 130-100-857

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	**2 mL CD133 MicroBeads – Tumor Tissue, human:** MicroBeads conjugated to monoclonal anti-human CD133 antibodies (isotype: mouse IgG1, clone AC133). **2 mL FcR Blocking Reagent, human:** Human IgG.
Capacity	For 10^9 total cells, up to 100 separations.
Product format	CD133 MicroBeads – Tumor Tissue 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 CD133$^+$ cells are magnetically labeled with CD133 MicroBeads – Tumor Tissue. Then, the cell suspension is loaded onto a MACS® Column, which is placed in the magnetic field of a MACS Separator. The magnetically labeled CD133$^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of CD133$^+$ cells. After removing the column from the magnetic field, the magnetically retained CD133$^+$ cells can be eluted as the positively selected cell fraction. To increase the purity, the positively selected cell fraction containing the CD133$^+$ cells must be separated over a second column.

1.2 Background information

The CD133 molecule is a 5-transmembrane cell surface antigen with a molecular weight of 117 kDa.[1] The CD133/1 (clone AC133) antibody recognizes an epitope of the CD133 antigen[2,3]. This epitope is called epitope 1 to distinguish it from another epitope (epitope 2) recognized by the clone 293C3.
CD133 has been found to be expressed on hematopoietic stem cells[1,2], circulating endothelial progenitor cells[4,5], and fetal neural stem cells[6,7] as well as on other tissue-specific stem cells, such as renal[8], prostate[9], and corneal[10] stem cells. In addition, CD133 was identified to be specifically expressed on cancer stem cells in multiple tumor entities like glioblastoma, lung cancer, prostate cancer, pancreatic cancer, and renal cancer[11]. In contrast to hematopoietic systems, where the epitopes of clones AC133 and 293C3 are co-expressed, only the epitope of clone AC133 is expressed in most of the analyzed tumor entities. Therefore, it is crucial to use only this clone if cells have to be identified or isolated. Furthermore, it was shown that the AC133 epitope but not the entire CD133 protein expression is lost upon CSC differentiation[12].

1.3 Applications

- Isolation or depletion of CD133$^+$ cells from non-hematopoietic origins (e.g. tumor tissue).

1.4 Reagent and instrument requirements

- MACS Columns and MACS Separators: CD133$^+$ cells can be enriched by using MS, or LS Columns or depleted with the use of LD Columns. For cells showing low expression levels of CD133, the use of an LS Column is recommended for optimal recovery during enrichment. Cells that strongly express the CD133 antigen can also be depleted using MS or LS Columns. Positive selection or depletion can also be performed by using the autoMACS® Pro or the autoMACS 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, autoMACS

▲ **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.

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- gentleMACS™ Dissociator (# 130-093-235), gentleMACS Octo Dissociator (# 130-095-937), or or gentleMACS Octo Dissociator with Heaters (# 130-096-427) for tissue dissociation when working with primary tissue.
- MACSmix™ Tube Rotator (# 130-090-753).
- Tumor Dissociation Kit, human (# 130-095-929).
- MACS Tissue Storage Solution (# 130-100-008).
- Labeling Check Reagent conjugated to, e.g., PE (# 130-095-228) for evaluation of MACS Separations by flow cytometry or fluorescence microscopy. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- 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. 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 Ca^{2+} or Mg^{2+} are not recommended for use.
- (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 solid tissue, prepare a single-cell suspension using manual methods or the gentleMACS Dissociator and tissue dissociation kits.

For details refer to the protocols section at www.miltenyibiotec.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).

As the epitopes of clone 293C3 and other clones are not co-expressed with the epitope of clone AC133 on the majority of tumor tissues, do not use those for the evaluation of your cell separation. Due to the low expression level on most cells it is also not possible to use AC133 fluorochrome conjugates for fluorescent staining of already MicroBead-labeled cells. For evaluation of MACS Separations by flow cytometry or fluorescence microscopy, use the Labeling Check Reagent conjugated to, e.g., PE (# 130-095-228).

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 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 60 µL of buffer per 10^7 total cells.
4. Add 20 µL of FcR Blocking Reagent per 10^7 total cells.
5. Add 20 µL of CD133 MicroBeads – Tumor Tissue per 10^7 total cells.
6. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C) under slow, continuous rotation using the MACSmix Tube Rotator.
7. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
8. (Optional) Add staining antibodies, e.g., 10 µL of Labeling Check Reagent-PE (# 130-095-228), mix well, and incubate for 5 minutes in the dark in the refrigerator (2–8 °C)

   ▲ **Note:** Labeling Check Reagent guarantees optimal flow cytometric analysis of isolated $CD133^+$ cells.
9. (Optional) Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
10. Resuspend up to 10^7 cells in 500 µL of buffer.

    ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
11. Proceed to magnetic separation (2.3).

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2.3 Magnetic separation

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of CD133$^+$ 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 only when the column reservoir is empty.
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 CD133$^+$ cells, enrich the eluted fraction 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.

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

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

▲ Buffers used for operating the autoMACS Pro Separator or the autoMACS 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® 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 the 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.

   Depletion: Depletes
   Collect negative fraction in row B of the tube rack.

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.

   Depletion: Depletes
   Collect negative fraction from outlet port neg1.

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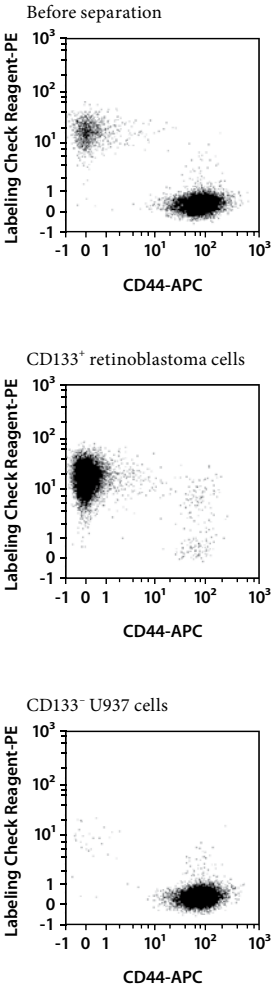
3. Example of a separation using the CD133 MicroBead Kit – Tumor Tissue

CD133$^+$ human retinoblastoma cells (WERI-Rb-1) were isolated from a mixture of U937 and WERI-Rb-1 cells using CD133 MicroBeads – Tumor Tissue, an MS Column, and an OctoMACS™ Separator. Cells were fluorescently stained with Labeling Check Reagent-PE (# 130-095-228) and CD44-APC (# 130-095-177) 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.



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All protocols and data sheets are available at **www.miltenyibiotec.com**.

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