CD133 MicroBead Kit - Tumor Tissue, human (92-01-0154)

[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.

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

[Product format] CD133 MicroBeads – Tumor Tissue are supplied as a suspension 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 labels.

[Principle]

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.

[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 Positive selection or depletion can also be performed by using the autoMACS Separator.
- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA .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 (FBS). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- Tube Rotator.
- Tumor Dissociation Kit, human.
- MACS Tissue Storage Solution.

[1.Sample preparation]

When working with solid tissue, prepare a single-cell suspension using manual methods or Dissociator and tissue dissociation kits.

▲ Note: Dead cells may bind non-specifically to MicroBeads. To remove dead cells, we recommend using density gradient centrifugation or the Dead Cell Removal Kit.

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.

[2. Magnetic labeling]

▲ Work fast, keep the 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 singlecell suspension before magnetic separation. Pass cells through 30 µm nylon mesh (Pre-Separation Filters) to remove cell clumps which may clog the column. Moisten filter with buffer before use.

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.

▲ 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.

6. Mix well and incubate for 15 minutes in the refrigerator (2-8 °C) under slow, continuous rotation using the Tube Rotator.
7. Wash cells by adding 1-2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Pipette off supernatant completely.
8. (Optional) Add staining antibodies, e.g., 10 µL of Labeling Check Reagent-PE, 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.

[3. Magnetic separation]

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of $CD133^+$ cells.

▲ 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.

2. Prepare column by rinsing with 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 flowthrough 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.

6. Pipette appropriate amount of buffer onto the column. Immediately flush out fraction with 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.
2. Prepare column by rinsing with 2 mL of buffer.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells which 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 Separator

1. Prepare and prime Separator.
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.