Miltenyi Biotec

Anti-XCR1 MicroBead Kit (Spleen)

mouse

Order no. 130-115-721

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 Anti-XCR1 MicroBeads, mouse:** MicroBeads conjugated to monoclonal anti-mouse XCR1 antibodies (isotype: recombinant human IgG1).
	2 mL Debris Removal MicroBeads
Capacity	For 2×10^9 total cells, up to 40 separations.
Product format	All reagents 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

Spleen can be dissociated into single-cell suspensions by combining mechanical dissociation with enzymatic digestion. The isolation of XCR1$^+$ cells is performed in a two-step procedure. First, the cell debris and dead cells of the single-cell suspension are magnetically labeled with Debris Removal MicroBeads. The labeled material is subsequently depleted by separation over a MACS® Column, which is placed in the magnetic field of a MACS Separator.
In the second step, the XCR1$^+$ cells are labeled with Anti-XCR1 MicroBeads and isolated by positive selection by separation over a MACS Column, which is placed in the magnetic field of a MACS Separator.
After removing the column from the magnetic field, the magnetically retained XCR1$^+$ cells can be eluted as the positively selected cell fraction. To increase the purity, the positively selected cell fraction containing the XCR1$^+$ cells must be separated over a second column.

Mouse splenocytes: Depletion of cell debris and dead cells

1. Magnetic labeling of cell debris and dead cells with Debris Removal MicroBeads.
2. Magnetic separation using an LD Column.

Positive selection of XCR1$^+$ cells

1. Magnetic labeling of XCR1 cells with Anti-XCR1 MicroBeads.
2. Magnetic separation using two MS Columns.

XCR1$^+$ cells

1.2 Background information

Cross-presenting CD8$^+$ conventional dendritic cells (cDCs) play a pivotal role in the induction of protective cytotoxic T lymphocyte (CTL) responses that are vital for the eradication of cancer and viral infections. In the past, studies of CD8$^+$ cDCs have been hampered by their scarcity and the lack of specific cell surface markers. Therefore, methods for the detection and isolation of these cells were commonly based on a multitude of immunophenotypic criteria, including the expression of CD11c and CD8 and the absence of CD3, CD4, SIRP-α, and CD11b. Recently, it was demonstrated that cross-presenting cDCs in lymphoid and non-lymphoid tissues specifically express the two receptors Clec9-α and XCR1. The expression of the latter has been correlated with the ability to take up and cross-present exogenous antigens.
The Anti-XCR1 MicroBead Kit (Spleen) is designed to isolate XCR1$^+$ cross-presenting dendritic cells (DCs) from mouse splenocytes.

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1.3 Applications

- Isolation of cross-presenting DCs from splenocytes for antigen uptake and antigen processing assays, T cell activation or T cell tolerance induction, cross-priming of cytotoxic T cells, or T helper cell polarization.

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

- MACS Columns and MACS Separators: Depletion of cell debris and dead cells can be performed on an LD Column. The subsequent positive selection of $XCR1^+$ cells can be performed on two MS Columns.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Depletion			
LD	10^8	5×10^8	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II, MultiMACS Cell24 Separator Plus
Positive selection			
MS	10^7	2×10^8	MiniMACS, OctoMACS, VarioMACS, SuperMACS II

▲ **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 antibodies for flow cytometric analysis, e.g., CD11c-VioBlue® or CD8a-APC. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- Pre-Separation Filters (70 µm) (# 130-095-823) to remove cell clumps.
- Spleen Dissociation Kit, mouse (# 130-095-926)
- gentleMACS™ Octo Dissociator with Heaters (# 130-096-427)

2. Protocol

2.1 Sample preparation

For the spleen dissociation use the gentleMACS Octo Dissociator with Heaters and the Spleen Dissociation Kit. For details refer to the respective data sheet and the gentleMACS Dissociator user manual.

2.2 Magnetic labeling of debris and dead cells

▲ 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 10^8 total cells. When working with fewer than 10^8 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 2×10^8 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 (70 µm), # 130-095-823) 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 450 µL of buffer per 10^8 total cells.
4. Add 50 µL of Debris Removal MicroBeads per 10^8 total cells.
5. Mix well and incubate for 5 minutes at room temperature (19–25 °C).
6. Proceed to magnetic separation (2.3).

2.3 Magnetic separation: Depletion of debris and dead cells

▲ Choose an LD Column and an appropriate MACS Separator. For details refer to the table in section 1.4.

▲ Always wait until the column reservoir is empty before proceeding to the next step.

1. Place LD Column in the magnetic field of a suitable MACS Separator. For details refer to 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 effluent; this is the unlabeled cell fraction. Perform washing steps by adding buffer two times. Only add new buffer when the column reservoir is empty.
5. Proceed to 2.4 for the labeling of $XCR1^+$ cells.

2.4 Magnetic labeling of $XCR1^+$ cells

▲ Volumes for magnetic labeling given below are for up to 5×10^7 total cells. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 450 µL of buffer per 5×10^7 total cells.
4. Add 50 µL of Anti-XCR1 MicroBeads per 5×10^7 total cells.

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5. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
6. Wash cells by adding 10 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. Resuspend up to 10^8 cells in 500 µL of buffer.

   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
8. Proceed to magnetic separation (2.5).

2.5 Magnetic separation: Positive selection of XCR1$^+$ cells

Positive selection with MS Columns

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

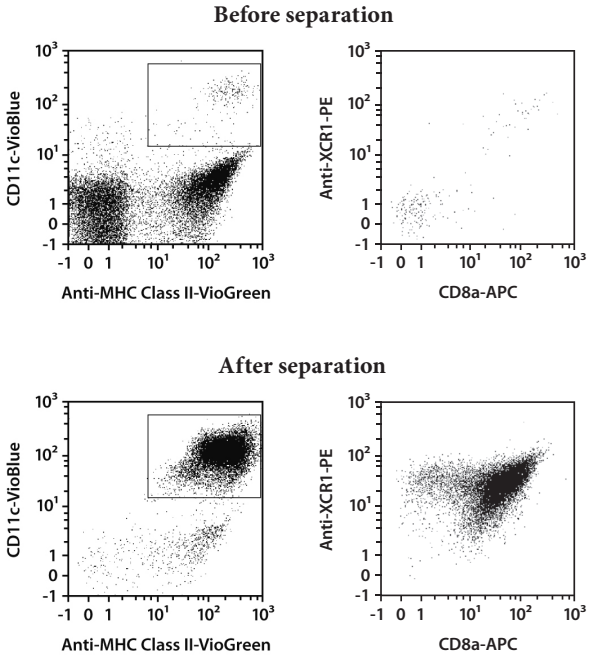
1. Place MS Column in the magnetic field of a suitable MACS Separator. For details refer to MS Column data sheet.
2. Prepare column by rinsing with 500 µL of buffer.
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.
4. Wash column with 3×500 µL of buffer. Collect unlabeled cells that pass through and combine with the effluent from step 3.

   ▲ **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.

   ▲ **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 1 mL of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.
7. To increase purity of XCR1$^+$ cells, the eluted fraction must be enriched over a second MS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

3. Example of a separation using the Anti-XCR1 MicroBead Kit (Spleen)

XCR1$^+$ DCs were isolated from a spleen single-cell suspension by using the Anti-XCR1 MicroBead Kit (Spleen) with one LD Column, two MS Columns, and a MiniMACS™ Separator. Cells were fluorescently stained with Anti-MHC Class II-VioGreen™, CD11c-VioBlue®, Anti-XCR1-PE, and CD8a-APC and analyzed by flow cytometry using the MACSQuant® Analyzer 10.
Cell debris, dead cells, and autofluorescent cells were exluded from analysis based on scatters and propidium iodide fluorescence. Dot plots on the right show conventional DCs gated on CD11c$^+$MHC class II$^+$ cells, stained for cross-presenting DC markers (CD8a and XCR1).



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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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