Miltenyi Biotec

# Rat CD43 MicroBeads

## for isolation of untouched B cells

2 ml Rat CD43 MicroBeads
For $10^9$ total cells

Order No. 130-090-742

## Contents


1. Description
   - 1.1 Principle of a MACS separation
   - 1.2 Reagent and instrument requirement
   - 1.3 Background and product applications
2. Protocol
   - 2.1 Sample preparation
   - 2.2 Magnetic labeling
   - 2.3 Magnetic separation
3. Example of a separation using Rat CD43 MicroBeads
4. References


## 1. Description

| | |
|---|---|
| **Component** | 2 ml Rat CD43 MicroBeads for $10^9$ total cells |
| **Specificity** | Antibody epitope of rat CD43 molecules |
| **Clone** | HIS17 |
| **Isotype** | Mouse IgG1 |
| **Product format** | The product is supplied as suspension containing 0.1 % stabilizer and 0.05 % sodium azide. |
| **Storage** | Store protected from light at 4°C. Do not freeze. The expiry date is indicated on the vial label. |

### 1.1 Principle of a MACS separation

For MACS separation, cells are magnetically labeled with Rat CD43 MicroBeads and passed through a separation column which is placed in the magnetic field of a MACS Separator. The magnetically labeled $CD43^+$ cells are retained in the column while the unlabeled $CD43^-$ cells run through. After removal of the column from the magnetic field, the magnetically retained $CD43^+$ cells can be eluted as magnetically labeled cell fraction.

### 1.2 Reagent and instrument requirement

- Buffer (degassed): phosphate buffered saline pH 7.2, supplemented with 0.5% bovine serum albumin (BSA) and 2 mM EDTA. Keep buffer cold (4°-8°C).

  ▲ **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 gelatine, human serum albumin, human serum, bovine serum. Buffers or media containing $Ca^{2+}$ or $Mg^{2+}$ are not recommended for use.
- (Optional) Pre-Separation Filter (# 130-041-407)
- Columns and separators:

| Column | max. number of labeled cells | max. number of total cells | Separator |
|---|---|---|---|
| **Positive selection** | | | |
| MS | $10^7$ | $2\times10^8$ | MiniMACS, OctoMACS, VarioMACS, SuperMACS |
| LS | $10^8$ | $2\times10^9$ | MidiMACS, QuadroMACS, VarioMACS, SuperMACS |
| **Depletion** | | | |
| LD | $10^8$ | $5\times10^8$ | MidiMACS, QuadroMACS, VarioMACS, SuperMACS |
| CS | $2\times10^8$ | | VarioMACS, SuperMACS |
| D | $10^9$ | | SuperMACS |
| **Positive selection or depletion** | | | |
| autoMACS | $2\times10^8$ | $4\times10^9$ | autoMACS |

### 1.3 Background and product applications

Rat CD43 MicroBeads are used for the isolation of untouched B cells from lymphoid tissue. The CD43 antigen (leukosialin)[1] is expressed on almost all rat leukocytes except resting peripheral B cells. After magnetic labeling with Rat CD43 MicroBeads, labeled non-B cells are retained on a separation column, whereas B lymphocytes remain untouched and are collected in the column effluent. CD43 is not consistently found on dendritic cells[2,3], NK cells[4] and resident peripheral macrophages[2,5]. CD43 expression can be induced by inflammation of synovial tissue.[6]
A highly pure population of B cells as obtained by using Rat CD43 MicroBeads can be used for cellular or molecular analyses of B cell differentiation and function.

## 2. Protocol

### 2.1 Sample preparation

▲ Prepare a single cell suspension from lymphoid or non-lymphoid tissue by standard preparation method (for details see General Protocols in MACS User Manuals or visit our website www.miltenyibiotec.com).

When working with spleen, centrifuge cell suspension at 300xg for 10 minutes and pipette off the supernatant. Prepare lysis buffer (0.15 M $NH_4Cl$, 10 mM $KHCO_3$, 0.1 mM EDTA). Lyse erythrocytes by adding lysis buffer directly to the cell pellet. Use 5 ml lysis buffer per 2-4 x $10^8$ total cells and incubate for 5 minutes at room temperature. Wash cells by adding about 10x the lysing volume of buffer. Centrifuge cells at 300xg for 10 minutes and resuspend cell pellet in buffer (see 2.2).



**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**

▲ For a proper performance it is important to obtain a single cell suspension before magnetic separation. To remove cell clumps which may clog the columns pass cells through 30 µm nylon mesh (Pre-Separation Filter # 130-041-407).

▲ Dead cells may bind nonspecifically to MACS MicroBeads. To remove dead cells, we recommend density gradient centrifugation using e.g. Ficoll-Paque™ or using the Dead Cell Removal Kit (# 130-090-101).



### 2.2 Magnetic labeling

▲ Avoid capping of antibodies on the cell surface during labeling and avoid nonspecific cell labeling. Therefore work fast, keep cells cold, use cold solutions only. Working on ice requires increased incubation times for MACS MicroBeads. Incubate in refrigerator at 4°-8°C.

1. Wash cells by adding 10-20x the labeling volume of buffer, centrifuge at 300xg for 10 minutes.
2. Pipette off the supernatant completely.
3. Resuspend cell pellet in 80 µl of buffer per $10^7$ **total** cells.
   ▲ **Note:** When working with lower cell numbers, use same volumes. When working with higher cell numbers, scale up all reagent volumes and total volumes, accordingly (e. g. for $2 \times 10^7$ total cells use twice the volume of all indicated reagent volumes and total volumes).
4. Add 20 µl of Rat CD43 MicroBeads per $10^7$ **total** cells.
   ▲ **Note:** Increased Rat CD43 MicroBead concentrations may lead to unspecific binding to non-target cells and may also impair antibody fluorochrome staining when using an antibody of the same specificity.
5. Mix well and incubate for 15 minutes at 4–8°C.
   ▲ **Note:** Higher temperatures and longer incubation times for magnetic labeling and staining may lead to unspecific cell labeling.
6. (Optional) Add fluorochrome-conjugated antibody at the titer recommended by the manufacturer and incubate for additional 5–10 minutes to evaluate the efficiency of the magnetic separation by flow cytometry or fluorescence microscopy.
7. Wash cells by adding 10–20x the labeling volume of buffer, centrifuge at 300 x g for 10 minutes.
8. Pipette off the supernatant.
9. Resuspend cell pellet in 500 µl buffer for up to $10^8$ **total** cells.
   ▲ **Note:** When working with higher cell numbers, scale up the buffer volume accordingly when applying cells onto separation column. Use maximum cell concentration of $10^8$ cells per 500 µl of buffer.
10. Proceed to magnetic separation.

### 2.3 Magnetic separation



#### Magnetic separation with MS and LS Columns

1. Choose a MS Column (up to $10^7$ magnetically labeled cells or up to $2 \times 10^8$ total cells) or a LS Column (up to $10^8$ magnetically labeled cells or up to $2 \times 10^9$ total cells).
   Place the column in the magnetic field of a suitable MACS Separator (see ”Column data sheets”).
2. Prepare column by rinsing with appropriate amount of buffer:
   MS: 500 µl LS: 3 ml.
3. Apply cell suspension in suitable amount of buffer onto the column:
   MS: 500–1000 µl LS: 1–10 ml.
   Allow the cells to pass through and collect effluent as fraction with unlabeled cells (untouched B cells).
4. Wash with appropriate amount of buffer:
   MS: 3 x 500 µl LS: 3 x 3 ml.
   Allow the cells to pass through and collect effluent as fraction with unlabeled cells (untouched B cells).
5. Remove column from separator and place column on a suitable collection tube.
6. Pipette appropriate amount of buffer onto the column:
   MS: 1 ml LS: 5 ml.
7. Firmly flush out magnetically labeled cells using the plunger supplied with the column.
   ▲ **Note:** To increase purity of the magnetically labeled cell fraction, it can be passed over a second, freshly prepared column.



#### Magnetic Separation with LD Columns

1. Choose a LD Column of up to $10^8$ magnetically labeled cells and up to $5 \times 10^8$ total cells.
   Place LD Column in the magnetic field of a suitable MACS Separator (see ”LD Column data sheet”).
2. Prepare column by rinsing with 2 ml of buffer.
3. Apply cell suspension in 500 µl buffer per $1.25 \times 10^8$ total cells on top of the column.
4. Collect the unlabeled cells which pass through and wash with 2 x 1 ml of buffer. Collect total effluent as unlabeled fraction.



#### Magnetic Separation with CS Columns

1. Assemble the CS Column and place it in the magnetic field of a suitable MACS Separator (see ”CS Column data sheet”).
2. Prepare column by filling and rinsing with 60 ml of buffer. Attach a 22G flow resistor to the 3-way-stopcock of the assembled column (see ”CS Column data sheet”).
3. Apply cell suspension in 500 µl buffer per $10^8$ total cells on top of the column.
4. Collect the unlabeled cells which pass through and wash with 30 ml buffer from top. Collect total effluent as unlabeled fraction.



#### Magnetic Separation with D Columns

Magnetic separation for up to $10^9$ magnetically labeled cells can be achieved by using D Columns. Place the column in a SuperMACS. For details on the column assembly and the separation, refer to the “D Column data sheet“.

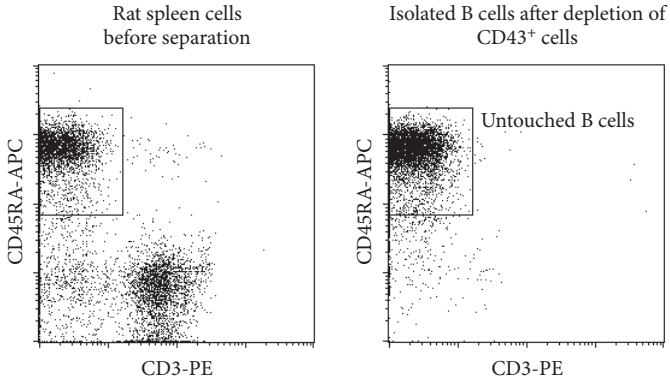


#### Magnetic separation with autoMACS

Positive selection or depletion of up to $2 \times 10^8$ magnetically labeled cells and up to $4 \times 10^9$ total cells can be achieved by using autoMACS. For the selection of the appropriate program please refer to the autoMACS User Manual.

## 3. Example for a separation of rat cells using Rat CD43 MicroBeads

Depletion of CD43$^+$ cells from a single cell suspension of rat spleen using Rat CD43 MicroBeads, MiniMACS and a MS Column. Cells are stained with CD45RA-APC (clone OX33) and CD3-PE (clone G4.18). Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.




## 4. References

1. Kroese, FGM; Wubbena, AS; Joling, P; Nieuwenhuis, P (1985) T lymphocytes in rat lymphoid follicles are a subset of T helper cells. Adv. Exp. Mol. Biol. 186: 443-449.
2. Xia, W; Schneeberger, EE; McCarthy, K; Kradin, R (1991) Accessory Cells of the Lung. II. Ia+ Pulmonary Dendritic Cells Display Cell Surface Antigen Heterogenity. Am. J. Respir. Cell. Mol. Biol. 5: 276-283.
3. Steiniger, B; Klempnauer, J; Wonigeit, K (1984) Phenotype and histological distribution of interstitial dendritic cells in the rat pancreas, liver, heart and kidney. Transplantation 38: 169-1752.
4. Cantrell, DA; Robins, RA; Brooks, CG; Baldwin, RW (1982) Phenotype of rat natural killer cells defined by monoclonal antibodies marking rat lymphocyte subsets. Immunology 45: 97-103.
5. Howell, DN; Ahjua, V; Jones, L; Blow, O; Sanfilippo, FP (1994) Differential expression of CD43 (leukosialin, sialophorin) by mononuclear phagocyte populations. J. Leukoc. Biol. 55: 536-544.
6. Volin, MV; Szekanecz, Z; Halloran, MM; Woods, JM; Magua, J; Damergis, JA Jr.; Haines, GK III, Crocker, PR; Koch, AE (1999) PECAM-1 and Leukosialin (CD43) expression correlate with heightened inflammation in rat adjuvant-induced arthritis. Exp. Mol. Pathol. 66: 211-219.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

### 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.

## Legal notices

### Limited product warranty

Miltenyi Biotec B.V. & Co. KG and/or its affiliate(s) warrant this product to be free from material defects in workmanship and materials and to conform substantially with Miltenyi Biotec's published specifications for the product at the time of order, under normal use and conditions in accordance with its applicable documentation, for a period beginning on the date of delivery of the product by Miltenyi Biotec or its authorized distributor and ending on the expiration date of the product's applicable shelf life stated on the product label, packaging or documentation (as applicable) or, in the absence thereof, ONE (1) YEAR from date of delivery ("Product Warranty"). Miltenyi Biotec's Product Warranty is provided subject to the warranty terms as set forth in Miltenyi Biotec's General Terms and Conditions for the Sale of Products and Services available on Miltenyi Biotec's website at www.miltenyibiotec.com, as in effect at the time of order ("Product Warranty"). Additional terms may apply. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.
THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING IF A PRODUCT IS SUITABLE FOR CUSTOMER'S PARTICULAR PURPOSE AND APPLICATION METHODS.

### Technical information

The technical information, data, protocols, and other statements provided by Miltenyi Biotec in this document are based on information, tests, or experience which Miltenyi Biotec believes to be reliable, but the accuracy or completeness of such information is not guaranteed. Such technical information and data are intended for persons with knowledge and technical skills sufficient to assess and apply their own informed judgment to the information. Miltenyi Biotec shall not be liable for any technical or editorial errors or omissions contained herein.
All information and specifications are subject to change without prior notice. Please contact Miltenyi Biotec Technical Support or visit www.miltenyibiotec.com for the most up-to-date information on Miltenyi Biotec products.

### Licenses

This product and/or its use may be covered by one or more pending or issued patents and/or may have certain limitations. Certain uses may be excluded by separate terms and conditions. Please contact your local Miltenyi Biotec representative or visit Miltenyi Biotec's website at www.miltenyibiotec.com for more information.
The purchase of this product conveys to the customer the non-transferable right to use the purchased amount of the product in research conducted by the customer (whether the customer is an academic or for-profit entity). This product may not be further sold. Additional terms and conditions (including the terms of a Limited Use Label License) may apply.
CUSTOMER'S USE OF THIS PRODUCT MAY REQUIRE ADDITIONAL LICENSES DEPENDING ON THE SPECIFIC APPLICATION. THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING FOR ITSELF WHETHER IT HAS ALL APPROPRIATE LICENSES IN PLACE. Miltenyi Biotec provides no warranty that customer's use of this product does not and will not infringe intellectual property rights owned by a third party. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

### Trademarks

autoMACS, MACS, MidiMACS, the Miltenyi Biotec logo, MiniMACS, OctoMACS, QuadroMACS, SuperMACS, and VarioMACS are registered trademarks or trademarks of Miltenyi Biotec and/or its affiliates in various countries worldwide. All other trademarks mentioned in this publication are the property of their respective owners and are used for identification purposes only.

Ficoll-Paque is a trademark of GE Healthcare companies.

Copyright © 2021 Miltenyi Biotec and/or its affiliates. All rights reserved.