CD294 (CRTH2) MicroBead Kit
human

Order no. 130-091-274

Index

1. **Description**
   - 1.1 **Principle of MACS® Separation**
   - 1.2 **Background and product applications**
   - 1.3 **Reagent and instrument requirements**
2. **Protocol**
   - 2.1 **Sample preparation**
   - 2.2 **Magnetic labeling of CD294 (CRTH2)$^+$ cells**
   - 2.3 **Magnetic separation of CD294 (CRTH2)$^+$ cells**
3. **Examples of a separation using the CD294 (CRTH2) MicroBead Kit**
   - 3.1 **Separation of CD294 (CRTH2)$^+$ cells**
   - 3.2 **Separation of CD4$^+$CD294 (CRTH2)$^+$ TH2 cells**
4. **References**

1. Description

Components	**2 mL FcR Blocking Reagent, human:** human IgG. **1 mL CD294 (CRTH2)-PE, human:** monoclonal anti-human CD294 (CRTH2) antibody conjugated to R-phycoerythrin (PE) (clone: BM16; isotype: rat IgG2a). **2 mL Anti-PE MicroBeads:** MicroBeads conjugated to monoclonal anti-PE antibody (isotype: mouse IgG1).
Size	For 10^9 total cells, up to 100 separations.
Product format	All components 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 MACS® Separation

First the CD294 (CRTH2)$^+$ cells are indirectly magnetically labeled with CD294 (CRTH2)-PE and Anti-PE 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 CD294 (CRTH2)$^+$ cells are retained on the column. The unlabeled cells run through. This cell fraction is depleted of CD294 (CRTH2)$^+$ cells. After removal of the column from the magnetic field, the magnetically retained CD294 (CRTH2)$^+$ cells can be eluted as the positively selected cell fraction. To achieve highest purities, the positively selected cell fraction containing the CD294 (CRTH2)$^+$ cells is separated over a second, new column.

1.2 Background and product applications

CD294 (CRTH2: chemoattractant receptor of TH2 cells) is a receptor for prostaglandin D_2. It is involved in lymphocyte migration. CD294 (CRTH2) is expressed on T helper type 2 (TH2) cells, a subpopulation of CD4$^+$ T cells, but is not present on T helper type 1 (TH1) cells.[1,2] The CD294 (CRTH2) antigen is highly expressed on peripheral blood basophils and eosinophils.[3] It is also expressed by a small population of CD8$^+$ T cells[1,2] and is discussed to be present on a subpopulation of dendritic cells[4].

Example applications

- Isolation or depletion of CD294 (CRTH2)$^+$ cells from peripheral blood mononuclear cells (PBMCs) or other single-cell preparations.
- For isolation of CD4$^+$CD294 (CRTH2)$^+$ TH2 cells from human PBMCs for further phenotypical characterization or functional analysis, the CD294 (CRTH2) MicroBead Kit can be used in combination with the CD4$^+$ T Cell Isolation Kit II (# 130-091-155). The isolation of CD4$^+$CD294 (CRTH2)$^+$ TH2 cells is performed in a two-step procedure. After depletion of non-CD4$^+$ T cells using the CD4$^+$ T Cell Isolation Kit II, the CD4$^+$CD294 (CRTH2)$^+$ TH2 cells are magnetically labeled and enriched by positive selection.

140-000-986.04

Miltenyi Biotec GmbH
Friedrich-Ebert-Str. 68
51429 Bergisch Gladbach, Germany
Phone +49-2204-8306-0 Fax +49-2204-85197

Miltenyi Biotec Inc.
12740 Earhart Avenue, Auburn CA 95602, USA
Phone 800 FOR MACS, 530 888-8871
Fax 530 888-8925

1.3 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 (4–8 °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 calf serum. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- MACS Columns and MACS Separators: Positive selection of CD294 $(CRTH2)^+$ cells is performed on two MS or LS Columns. Depletion is done with LD Columns. Depletion and positive selection can also be performed by using the autoMACS™ Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Positive selection			
MS	10^7	2×10^8	MiniMACS, OctoMACS, VarioMACS, SuperMACS
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
Depletion			
LD	10^8	5×10^8	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
Positive selection or depletion			
autoMACS	2×10^8	4×10^9	autoMACS

▲ **Note:** Column adapters are required to insert certain columns into VarioMACS™ or SuperMACS™ Separator. For details, see MACS Separator data sheets.

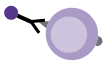
- (Optional) $CD4^+$ T Cell Isolation Kit II (# 130-091-155).
- (Optional) Additional staining reagents, such as CD4-FITC (# 130-080-501) or CD4-APC (# 130-091-232).
- (Optional) Propidium iodide (PI) or 7-AAD for flow cytometric exclusion of dead cells.
- (Optional) Pre-Separation Filters (# 130-041-407) 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, e.g. using Ficoll-Paque™. For details see section General Protocols in the User Manuals or visit www.miltenyibiotec.com/protocols.

▲ **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.



2.2 Magnetic labeling of CD294 $(CRTH2)^+$ 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 10^7 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 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 separation. Pass cells through 30 µm nylon mesh (Pre-Separation Filters, # 130-041-407) to remove cell clumps which may clog the column.

▲ Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

1. Determine cell number.
2. Centrifuge the cells at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 80 µL of buffer.
4. Add 20 µL of **FcR Blocking Reagent** and 10 µL of **CD294 (CRTH2)-PE** per 10^7 cells.
5. (Optional) Add additional staining antibodies, e.g. 10 µL of CD4-FITC (# 130-080-501) or CD4-APC (# 130-091-232).

   ▲ **Note:** Do not use tandem conjugates of phycoerythrin, like Cy-Chrome™ (Becton Dickinson), PE-Cy5 (Serotec), ECD, PC5 (Coulter-Immunotech) etc., they may also be recognized by the Anti-PE MicroBeads.
6. Mix well and refrigerate for 10 minutes (4–8 °C).
7. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
8. Resuspend cell pellet in 80 µL of buffer per 10^7 cells.
9. Add 20 µL of **Anti-PE MicroBeads** per 10^7 cells.
10. Mix well and refrigerate for additional 15 minutes (4–8 °C).
11. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
12. Resuspend up to 10^8 cells in 500 µL of buffer.

    ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.

    ▲ **Note:** For depletion with LD Columns, resuspend cell pellet in 500 µL of buffer for up to 1.25×10^8 cells.
13. Proceed to magnetic separation (2.3).

140-000-986.04

2.3 Magnetic separation: Positive selection of CD294 (CRTH2)$^+$ cells

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of CD294 (CRTH2)$^+$ cells. For details see table in section 1.3.

Positive selection with MS or LS Columns

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

1. Place column in the magnetic field of a suitable MACS Separator. For details see MACS Column data sheets.
2. Prepare column by rinsing with appropriate amount of buffer:
   MS: 500 µL LS: 3 mL
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with appropriate amount of buffer. Perform washing steps by adding buffer three times. Only add new buffer when the column reservoir is empty.
   MS: 3×500 µL LS: 3×3 mL
   Collect total effluent; this is the unlabeled cell fraction.
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 appropiate amount of buffer onto the column. Immediately flush out the fraction with magnetically labeled cells (CD294 (CRTH2)$^+$ cells) by firmly applying the plunger supplied with the column.
   MS: 1 mL LS: 5 mL
7. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

Depletion of CD294 (CRTH2)$^+$ cells with LD Columns

1. Place LD Column in the magnetic field of a suitable MACS Separator. For details see 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 which pass through and wash column with 2×1 mL of buffer. Collect total effluent. This is the depleted, CD294 (CRTH2)$^-$ cell fraction.

Magnetic separation of CD294 (CRTH2)$^+$ cells with the autoMACS™ Separator

▲ Refer to the autoMACS™ User Manual for instructions on how to use the autoMACS Separator.

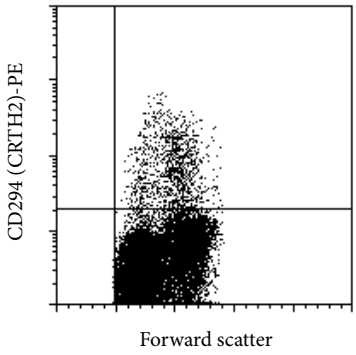
1. Prepare and prime autoMACS Separator.
2. Place tube containing the magnetically labeled cells in the autoMACS Separator. For a standard separation, choose one of the following separation programs:
   Positive selection: "Posseld"
   Depletion: "Depletes"
   ▲ **Note:** Program choice depends on the isolation strategy, the strength of magnetic labeling and the frequency of magnetically labeled cells. For details see autoMACS User Manual: "autoMACS Cell Separation Programs".
3. When using the program "Posseld", collect positive fraction from outlet port pos2. This is the purified CD294 (CRTH2)$^+$ cell fraction.
   When using the program "Depletes", collect unlabeled fraction from outlet port neg1. This is the CD294 (CRTH2)$^-$ cell fraction.

3. Examples of a separation using the CD294 (CRTH2) MicroBead Kit

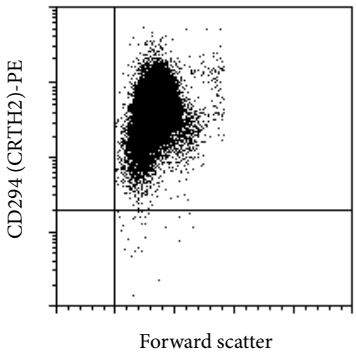
3.1 Separation of CD294 (CRTH2)$^+$ cells

CD294 (CRTH2)$^+$ cells were isolated from human PBMCs by using the CD294 (CRTH2) MicroBead Kit, two MS Columns and a MiniMACS™ Separator. The cells are fluorescently stained with CD294 (CRTH2)-PE. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

PBMCs before separation



CD294 (CRTH2)$^+$ cell fraction

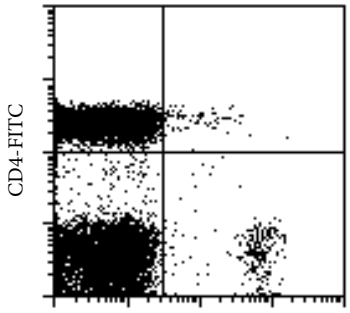


141-000-986.04

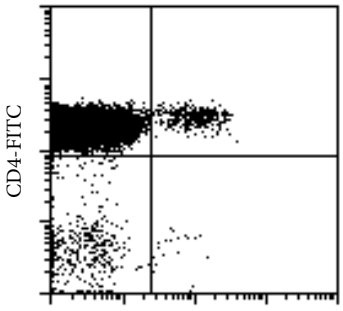
3.2 Separation of CD4$^+$CD294 (CRTH2)$^+$ Th2 cells

CD4$^+$CD294 (CRTH2)$^+$ Th2 cells were isolated from human PBMCs by using the CD4$^+$ T Cell Isolation Kit II (# 130-091-155) and CD294 (CRTH2) MicroBead Kit, an LD, and two MS Columns, a MidiMACS™ Separator and a MiniMACS™ Separator. The cells are fluorescently stained with CD294 (CRTH2)-PE and CD4-FITC. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

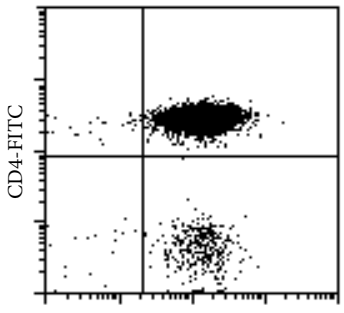
PBMCs before separation



Pre-enriched CD4$^+$ T cells after depletion of non-CD4$^+$ cells



Enriched CD4$^+$CD294 (CRTH2)$^+$ Th2 cells



4. References

1. Nagata, K. *et al.* (1999) Selective expression of a novel surface molecule by human Th2 cells *in vivo*. J. Immunol. 162: 1278–1286.
2. Cosmi, L. *et al.* (2000) CRTH2 is the most reliable marker for the detection of circulating human type 2 Th and type 2 cytotoxic cells in health and disease. Eur. J. Immunol. 30: 2972–2979. [1713}
3. Nagata, K. *et al.* (1999) CRTH2, an orphan receptor of T-helper-2-cells, is expressed on basophils and eosinophils and responds to mast cell-derived factor(s). FEBS Lett. 459: 195–199.
4. Messi, M. *et al.* (2003) Memory and flexibility of cytokine gene expression as separable properties of human Th1 and Th2 lymphocytes. Nat. Immunol. 4: 78–86.

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.

Warranty

The products sold hereunder are warranted only to be free from defects in workmanship and material at the time of delivery to the customer. Miltenyi Biotec GmbH makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the technical specifications of the products. Miltenyi Biotec GmbH's liability is limited to either replacement of the products or refund of the purchase price. Miltenyi Biotec GmbH is not liable for any property damage, personal injury or economic loss caused by the product.

140-000-986.04