Miltenyi Biotec

CD294 (CRTH2) antibodies
human

CD294 (CRTH2)-PE	130-091-238
CD294 (CRTH2)-Biotin	130-091-239

Index

1. Description

Clone	BM16 (isotype: rat IgG2a).
Product format	1 mL CD294 (CRTH2) antibodies, human: monoclonal anti-human CD294 (CRTH2) antibodies conjugated to R-phycoerythrin (**PE**), or to biotin (**Biotin**). The antibodies are supplied in a solution containing stabilizer and 0.05% sodium azide.
Product size	100 tests (for up to 10^9 total cells).
Storage	Store protected from light at 4–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Background and product applications

The CD294 (CRTH2: chemoattractant receptor of TH2 cells) is a receptor for prostaglandin D_2, and is involved in migration of leukocytes. 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] Also, 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].

Product applications

- Identification and enumeration of $CD294\ (CRTH2)^+$ cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® separations by flow cytometry or fluorescence microscopy.

1.2 Examples of staining concentrations

for human cells.

CD294 (CRTH2) conjugate	PE	Biotin
	Recommended antibody dilution	
Flow cytometry[a]		
- in general	1:11	1:11
- formaldehyde-fixed cells	1:11	1:11

a) Given antibody dilutions are for a cell concentration of up to 1×10^8 cells/mL buffer.

1.3 Reagent requirements

- Buffer: Prepare a solution containing PBS (phosphate buffered saline) pH 7.2, 0.5% BSA and 2 mM EDTA, e.g. by diluting MACS® BSA Stock Solution (# 130-091-376) 1:20 with autoMACS™ Rinsing Solution (# 130-091-222). 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 human serum albumin, human serum or fetal calf serum. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.
- FcR Blocking Reagent (# 130-059-901) to avoid Fc receptor-mediated cell staining.
- (Optional) Additional staining antibodies such as CD4-FITC (# 130-080-501) or CD123-APC (# 130-090-901).
- (Optional) Ant-Biotin-FITC (# 130-090-857), Anti-Biotin-PE (# 130-090-756), or Anti-Biotin-APC (# 130-090-856) as secondary antibody reagent in combination with CD294 (CRTH2)-Biotin.
- (Optional) PI (propidium iodide) or 7-AAD for flow cytometric exclusion of dead cells without cell fixation. For cell fixation and flow cytometric exclusion of dead cells, the Fixation and Dead Cell Discrimination Kit (# 130-091-163) is recommended.

2. General protocol for immunofluorescent staining

▲ Volumes for fluorescent labeling given below are for **up to** 10^7 nucleated 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 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Resuspend up to 10^7 nucleated cells per 100 µL of buffer.
2. Add 20 µL FcR Blocking Reagent and 10 µL of CD294 (CRTH2) antibodies.
3. (Optional) Add additional staining antibodies, e.g. 10 µL of CD4-FITC (# 130-080-501) or CD123-APC (# 130-090-901).
4. Mix well and incubate for 10 minutes in the dark at 4–8 °C.

   ▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.
5. 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.
6. (Optional) If CD294 (CRTH2)-Biotin was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL of Anti-Biotin antibody (Anti-Biotin-FITC # 130-090-857, Anti-Biotin-PE # 130-090-756, or Anti-Biotin-APC # 130-090-856), and continue as described in step 4 and 5.
7. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

140-000-987.03

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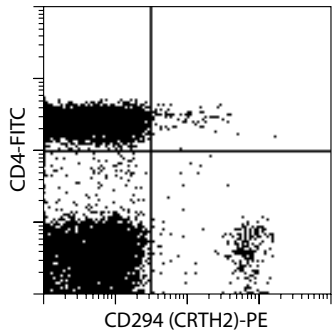
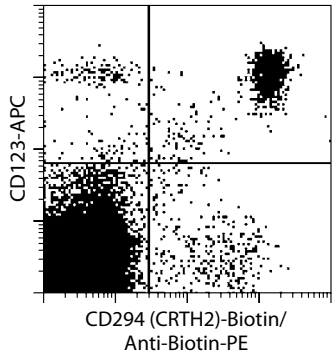
3. Examples of immunofluorescent staining with CD294 (CRTH2) antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with CD4-FITC and CD294 (CRTH2)-PE (a) or CD123-APC and CD294 (CRTH2)-Biotin and Anti-Biotin-PE (b), and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

(a) Human PBMCs stained with CD294 (CRTH2)-PE and CD4-FITC.



(b) Human PBMCs stained with CD294 (CRTH2)-Biotin/Anti-Biotin-PE and CD123-APC.



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 T_H1 and T_H2 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.

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