

CD294 (CRTH2) antibodies, human

For research use only

One test corresponds to labeling of up to 10^7 cells in a total volume of 100 μ L.

Product	Content	Order no.
CD294 (CRTH2)-PE	for 30 tests	130-109-345
CD294 (CRTH2)-PE	for 100 tests	130-109-267
CD294 (CRTH2)-APC	for 30 tests	130-109-346
CD294 (CRTH2)-APC	for 100 tests	130-109-268
CD294 (CRTH2)-PE-Vio770	for 30 tests	130-109-347
CD294 (CRTH2)-PE-Vio770	for 100 tests	130-109-269
CD294 (CRTH2)-APC-Vio770	for 30 tests	130-109-348
CD294 (CRTH2)-APC-Vio770	for 100 tests	130-109-270
CD294 (CRTH2)-Biotin	for 30 tests	130-109-344
CD294 (CRTH2)-Biotin	for 100 tests	130-109-266

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.

Technical data and background information

Antigen	CD294 (CRTH2)
Clone	REA598
Isotype	recombinant human IgG1
Isotype control	REA Control (S) antibodies
Alternative names of antigen	CRTH2
Molecular mass of antigen [kDa]	43
Distribution of antigen	T helper cells, basophils, eosinophils, T cells, dendritic cells
Product format	Reagents are supplied in buffer containing stabilizer and 0.05% sodium azide.
Fixation	Cells should be stained prior to fixation, if formaldehyde is used as a fixative.
Storage	Store protected from light at 2–8 °C. Do not freeze.

Clone REA598 recognizes the CD294 antigen, also known as chemoattractant receptor-homologous molecule expressed on TH2 cells (CRTH2). CD294 is a seven-transmembrane G protein-coupled receptor for prostaglandin D2 involved in migration of leukocytes and is expressed on T helper type 2 (TH2) cells, a subpopulation of CD4⁺ T cells, but not on TH1 cells. The CD294 antigen is highly

expressed on peripheral blood basophils and eosinophils. It is also present on a small population of CD8⁺ T cells and on a subpopulation of dendritic cells.

Additional information: Clone REA598 displays negligible binding to Fc receptors.

Reagent requirements

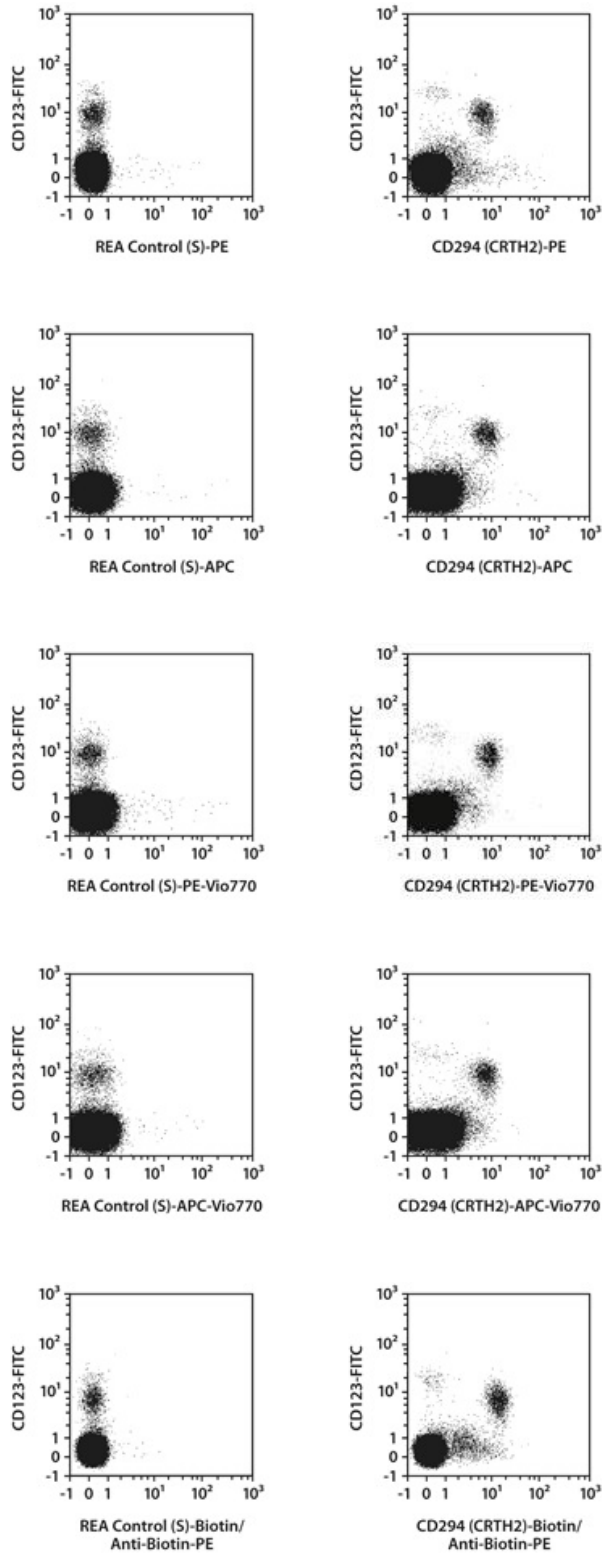
- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS[®] BSA Stock Solution (# 130-091-376) 1:20 with autoMACS[®] Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C).
Note: EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- (Optional) Fluorochrome-conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with biotinylated antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) for flow cytometric exclusion of dead cells without fixation.
- (Optional) Fixation and Dead Cell Discrimination Kit (# 130-091-163) for cell fixation and flow cytometric exclusion of dead cells.

Protocol for cell surface staining

- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:11 for up to 10⁷ cells/100 µL of buffer.
 - Volumes given below are for up to 10⁷ nucleated cells. When working with fewer than 10⁷ 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 2×10⁷ nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).
1. Determine cell number.
 2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
 3. Resuspend up to 10⁷ nucleated cells per 100 µL of buffer.
 4. Add 10 µL of the antibody.
 5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).
Note: Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.
 6. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
 7. (Optional) If biotinylated antibody was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL of fluorochrome-conjugated anti-biotin antibody, and continue as described in steps 5 and 6.
 8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

Examples of immunofluorescent staining

Human peripheral blood mononuclear cells (PBMCs) were stained with CD294 (CRTH2) antibodies or with the corresponding REA Control (S) antibodies (left image) as well as with CD123 antibodies. Flow cytometry was performed using the MACSQuant[®] Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



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. 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.
3. 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.

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.

Miltenyi Biotec GmbH | Friedrich-Ebert-Straße 68 | 51429 Bergisch Gladbach | Germany | Phone +49 2204 8306-0 | Fax +49 2204 85197 | macs@miltenyibiotec.de | www.miltenyibiotec.com

Miltenyi Biotec provides products and services worldwide. Visit www.miltenyibiotec.com/local to find your nearest Miltenyi Biotec contact.

Unless otherwise specifically indicated, Miltenyi Biotec products and services are for research use only and not for therapeutic or diagnostic use. autoMACS, MACS, MACSQuant, Vio, VioBlue, VioBright, and VioGreen are registered trademarks or trademarks of Miltenyi Biotec GmbH and/or its affiliates in various countries worldwide. Copyright © 2018 Miltenyi Biotec GmbH and/or its affiliates. All rights reserved.