Miltenyi Biotec

Anti-CCR10 antibodies
human

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 Monoclonal Anti-CCR10 antibodies, human conjugated to:

Conjugate	Order no. 1 mL (100 tests)	Order no. 300 µL (30 tests)
PE	130-104-822	130-104-868
APC	130-104-821	130-104-867
Biotin	130-104-820	130-104-866

Clone REA326 (isotype control : REA Control (S)).

Capacity 1 mL: 100 tests or up to 10^9 total cells

300 µL: 30 tests or up to 3×10^8 total cells.

Product format Antibodies 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 Background information

- Antigen: CCR10
- Synonym: C-C chemokine receptor type 10 (C-C CKR-10, CC-CKR-10, CCR-10); G-protein coupled receptor 2 (GPR2, GPR-2)
- Expression patterns: Clone REA326 recognizes the human C-C chemokine receptor type 10 (CCR10) antigen, a multi-pass membrane protein which is also known as G-protein coupled receptor 2 (GPR2). CCR10 is expressed by various subsets of innate-like T cells that are programmed to localize to the skin during their developmental processes in the thymus. Circulating T cells might be imprinted by skin-associated antigen-presenting cells to express CCR10 for their recruitment to the skin during the local immune response. On the other hand, IgA antibody-producing B cells generated in mucosa-associated lymphoid tissues express CCR10 for their migration and maintenance at mucosal sites. CCR10 and its ligands chemokines CCL27 and CCL28 are uniquely involved in the epithelial immunity. CCL27 is up-regulated in inflamed skin, whereas CCL28 is selectively expressed in intestinal epithelium. In vitro, CCL27–CCR10 interactions mediate the preferential migration of skin-homing CLA-bearing memory T cells. Increasing evidence also found that CCR10/ligands are involved in regulation of other immune cells in epithelial immunity and are frequently exploited by epithelium-localizing or -originated cancer cells for their survival, proliferation and evasion from immune surveillance. Additional information: Clone REA313 displays negligible binding to Fc receptors.

1.2 Applications

- Identification and enumeration of $CCR10^+$ cells by flow cytometry.

1.3 Recommended antibody dilution

The recommended antibody dilution for all Anti-CCR10 conjugates is **1:11 for up to 10^7 cells/100 µL** of buffer for labeling of cells and subsequent analysis by flow cytometry.

Cells should be stained prior to fixation, if formaldehyde is used as a fixative.

1.4 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 Ca^{2+} or Mg^{2+} are not recommended for use.

- (Optional) Conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with Anti-CCR10-Biotin.
- (Optional) For antibodies for additional staining or for isotype control, refer to www.miltenyibiotec.com/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

140-004-410.01

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 Inc.
2303 Lindbergh Street, Auburn, CA 95602, USA
Phone 800 FOR MACS, +1 530 888 8871, Fax +1 530 888 8925
macs@miltenyibiotec.com

of dead cells.

2. General protocol for immunofluorescent staining

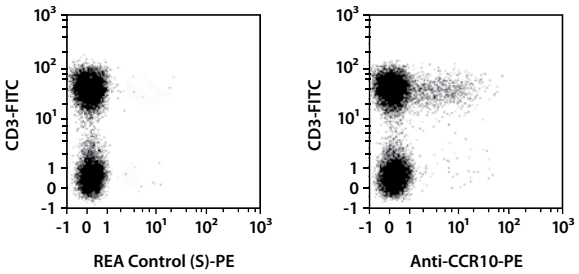
Volumes given below are for **up to 10^7** nucleated cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^7 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^7 nucleated cells per 100 µL of buffer.
4. Add 10 µL of the Anti-CCR10 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 Anti-CCR10-Biotin was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL of 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.

3. Example of immunofluorescent staining with Anti-CCR10 antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with Anti-CCR10 antibodies conjugated to PE or with the corresponding REA control (S) antibodies (left image), as well as with CD3-FITC and analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



4. References

1. Jarmin, D. I. *et al.* (2000) Cutting edge: identification of the orphan receptor G-protein-coupled receptor 2 as CCR10, a specific receptor for the chemokine ESkine J. Immunol. 164 (7): 3460–3464.
2. Sisirak, V. *et al.* (2011) CCR6/CCR10-mediated plasmacytoid dendritic cell recruitment to inflamed epithelia after instruction in lymphoid tissues Blood 118 (19): 5130–5140.
3. Homey, B. *et al.* (2002) CCL27-CCR10 interactions regulate T cell-mediated skin inflammation Nat. Med. 8 (2): 157–165.
4. Xiong, N. (2012) CCR10 and its ligands in regulation of epithelial immunity and diseases Protein Cell 3 (8): 571–580.

Refer to www.miltenyibiotec.com for all data sheets and protocols.

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.

140-004-410.01