

CD197 (CCR7)-PE	130-093-621
CD197 (CCR7)-APC	130-093-624
CD197 (CCR7) pure	130-093-626

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1. Description

Components	1 mL CD197 (CCR7) antibodies, human: monoclonal CD197 (CCR7) antibodies conjugated to R-phycoerythrin (PE) or allophycocyanin (APC). The unconjugated (pure) antibody is supplied at a concentration of 100 µg/mL.
Clone	FR11-11E8 (isotype: mouse IgG1).
Capacity	100 tests or up to 10 ⁹ 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

Chemokine (C-C motif) receptor 7 (CCR7), designated as CD197, is a chemokine receptor that mediates homing of T cells to secondary lymphoid organs via high endothelial venules (HEV). For example, naive T cells migrate very efficiently through lymph nodes using the adhesion molecule L-selectin, CD62L, and CCR7. Ligands for CCR7, such as CCL19, are expressed by the HEV of secondary lymphoid organs, by parenchymal cells within T cell zones of lymph nodes, and by endothelial cells at the openings of lymphatic vessels within peripheral tissues. Expression of CCR7 and the CD45RA isoform distinguishes three subsets of T cells: naive T cells (CCR7⁺CD45RA⁺), central memory T cells (CCR7⁺CD45RA⁻), and effector memory T cells (CCR7⁻CD45RA⁻).¹

1.2 Application

- Identification and enumeration of CCR7⁺ cells by flow cytometry or fluorescence microscopy.

1.3 Recommended antibody dilution

For antibody labeling of human cells.

CD197 (CCR7) conjugate	PE	APC
Flow cytometry^a		
- In general	1:11	1:11
- Formaldehyde-fixed cells ^b	1:11	1:11

a) Given antibody dilutions are for a cell concentration of up to 10⁷ cells/100 µL of buffer.
b) For optimal results, cells must be stained prior to fixation.

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). BSA can be replaced by other proteins such as human serum albumin, human serum, or fetal bovine serum. Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- (Optional) FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor-mediated antibody labeling.
- (Optional) Anti-PE MicroBeads (# 130-048-801) or Anti-APC MicroBeads (# 130-090-855).
- (Optional) CD4-FITC (# 130-808-501), CD4-PE (# 130-091-231), CD45RA-FITC (# 130-092-247), or CD45RA-PE (# 130-092-248). For more information about fluorochrome-conjugated antibodies see www.miltenyibiotec.com.
- (Optional) Mouse IgG1-PE (# 130-092-212) or Mouse IgG1-APC (# 130-092-214) for isotype control.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD 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.

2. General protocol for immunofluorescent staining

▲ 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.

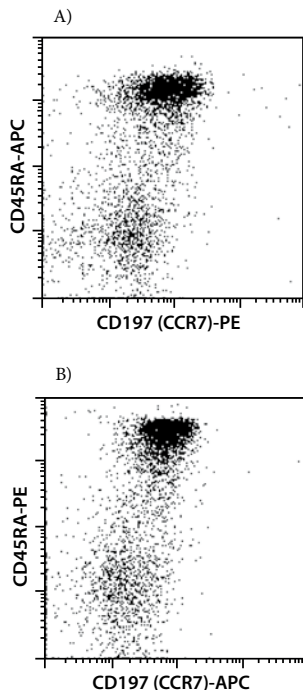
4. Add 10 μ L of the CD197 (CCR7) antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).

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

6. Wash cells by adding 1–2 mL of buffer and centrifuge at 300 \times g for 10 minutes. Aspirate supernatant completely.
7. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

3. Examples of immunofluorescent staining with CD197 (CCR7) antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with CD197 (CCR7) antibodies conjugated to PE (A) or APC (B), CD4-FITC, and CD45RA-APC or CD45RA-PE. Cells were analyzed by flow cytometry and gated on CD4⁺ T cells. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



4. Reference

1. Sallusto, F. *et al.* (1999) Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature* 401: 708–712.

All protocols and data sheets are available at www.miltenyibiotec.com.

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

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