

## Reagents Provided

**Peridinin-Chlorophyll-Protein-Complex (PerCP)-conjugated mouse monoclonal anti-human CCR7:** Supplied as 50 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

**Clone #:** 150503

**Isotype:** mouse IgG<sub>2A</sub>

## Reagents Not Provided

- PBS (Dulbecco's PBS)
- BSA

## Storage

Reagents are stable for **twelve months** from date of receipt when stored in the dark at 2° - 8° C.

## Intended Use

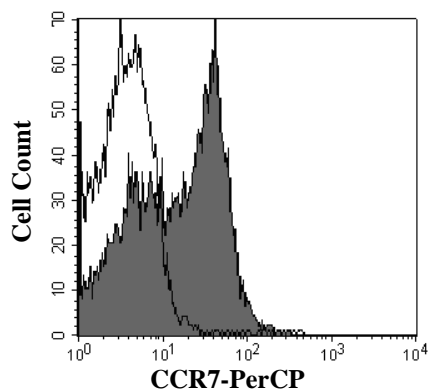
Designed to quantitatively determine the percentage of cells bearing CCR7 within a population and qualitatively determine the density of CCR7 on cell surfaces by flow cytometry.

## Principle of the Test


Washed cells are incubated with the PerCP-labeled monoclonal antibody, which binds to cells expressing CCR7. Unbound PerCP-conjugated antibody is then washed from the cells. Cells expressing CCR7 are fluorescently stained, with the intensity of staining directly proportional to the density of expression of CCR7. Cell surface expression of CCR7 is determined by flow cytometry. PerCP has a maximum absorption of 482 nm and 564 nm and a maximum emission of 675 nm.

## Reagent Preparation

**PerCP-conjugated mouse anti-human CCR7:** Use as is; no preparation necessary.



Human lymphocytes were stained with PerCP-conjugated anti-human CCR7 (Catalog # FAB197C, filled histogram) or isotype control (Catalog # IC003C, open histogram).

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FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

## Sample Preparation

**Peripheral blood cells:** Whole blood should be collected in evacuated tubes containing EDTA or heparin as the anticoagulant. Contaminating serum components should be removed by washing the cells three times in an isotonic phosphate buffer (supplemented with 0.5% BSA) followed by centrifugation at 500 x g for 5 minutes. 50 µL of packed cells should then be transferred to a 5 mL tube for staining with the monoclonal antibody. Whole blood will require lysis of RBC following the staining procedure.

**Cell Cultures:** Continuous cell lines or activated cell cultures should be centrifuged at 500 x g for 5 minutes and washed three times in an isotonic PBS buffer (supplemented with 0.5% BSA) to remove any residual growth factors that may be present in the culture medium. Cells should then be resuspended in the same buffer to a final concentration of 4 x 10<sup>6</sup> cells/mL and 25 µL of cells (1 x 10<sup>5</sup>) transferred to a 5 mL tube for staining.

Note: Adherent cell lines may require pretreatment with 0.5 mM EDTA to facilitate removal from their substrates. Cells that require trypsinization to enable removal from their substrates should be further incubated in medium for 6 - 10 hours on a rocker platform to enable regeneration of the receptors. The use of the rocker platform will prevent reattachment to the substrate.

## Sample Staining

- 1) Cells should be Fc-blocked by treatment with 1 µg of human IgG/10<sup>5</sup> cells for 15 minutes at room temperature prior to staining. Do not wash excess blocking IgG from this reaction.
- 2) Transfer 25 µL of the Fc-blocked cells (1 x 10<sup>5</sup> cells) or 50 µL of packed whole blood to a 5 mL tube.
- 3) Add 10 µL of PerCP-conjugated CCR7 reagent.
- 4) Incubate for 30 - 45 minutes at 2° - 8° C.
- 5) Following this incubation, remove unreacted CCR7 reagent by washing the cells twice in 4 mL of the same PBS buffer (*note: whole blood will require an RBC lysis step at this point using any commercially available lysing reagent, such as R&D Systems Whole Blood Lysing Kit, Catalog # WL1000*).
- 6) Finally, resuspend the cells in 200 - 400 µL of PBS buffer for analysis by flow cytometry.
- 7) As a control for analysis, cells in a separate tube should be treated with PerCP-labeled mouse IgG<sub>2A</sub> antibody.

This procedure may need modification, depending upon final utilization.

## Background Information

The human CCR7 chemokine receptor is a member of the rhodopsin superfamily of G-linked receptors that span the cell membrane with seven hydrophobic transmembrane domains. CCR7, originally identified as the EBV-induced gene-1,<sup>1</sup> was later characterized as the receptor for two chemokines, CCL19 (MIP-3 $\beta$ , Exodus-3, ELC) and CCL21 (SLC, 6CKine, Exodus-2).<sup>2-4</sup> CCR7 expression on T cells and mature dendritic cells is consistent with the chemotactic action of CCL19 and CCL21 on these two cell types.<sup>3,5,6</sup> Both memory and naive T cells express CCR7.<sup>7</sup> CCR7 also appears to be a useful marker in distinguishing between CCR7<sup>-</sup> effector memory T cells with full effector function and CCR7<sup>+</sup> central memory T cells which require a secondary stimulus prior to displaying effector functions.<sup>7</sup> Overall, CCR7 and its two ligands appear to be involved in the recruitment and retention of cells to secondary lymphoid organs. This idea is supported by studies with CCR7 deficient animals, which display irregular distribution of both T and B cells within lymph nodes, Peyer's patches and in splenic periarteriolar lymphoid sheaths.<sup>8</sup>

## References

1. Birkenbach, M. *et al.* (1993) *J. Virol.* **67**:2209.
2. Rossi, D.L. *et al.* (1997) *J. Immunol.* **158**:1033.
3. Yoshida, R. *et al.* (1997) *J. Biol. Chem.* **272**:13803.
4. Yoshida, R. *et al.* (1998) *J. Biol. Chem.* **273**:7118.
5. Sallusto, F. *et al.* (1998) *Eur. J. Immunol.* **28**:2760.
6. Willimann, K. *et al.* (1998) *Eur. J. Immunol.* **28**:2025.
7. Sallusto, F. *et al.* (1999) *Nature* **401**:708.
8. Forster, R. *et al.* (1999) *Cell* **99**:23.

**Warning:** Contains sodium azide as a preservative - sodium azide may react with lead and copper plumbing to form explosive metal azides. Flush with large volumes of water during disposal.