

Reagents Provided

Peridinin-Chlorophyll-Protein-Complex (PerCP)-conjugated mouse monoclonal anti-human CCR2: Supplied as 50 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 48607

Isotype: mouse IgG_{2B}

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

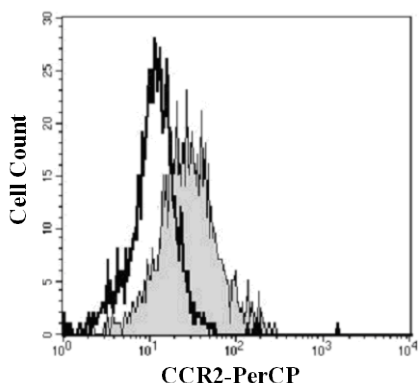
Designed to quantitatively determine the percentage of cells bearing CCR2 within a population and qualitatively determine the density of CCR2 on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the PerCP-labeled monoclonal antibody, which binds to cells expressing CCR2. Unbound PerCP-conjugated antibody is then washed from the cells. Cells expressing CCR2 are fluorescently stained, with the intensity of staining directly proportional to the density of expression of CCR2. Cell surface expression of CCR2 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 CCR2: Use as is; no preparation necessary.



Human monocytes were stained with PerCP-conjugated anti-human CCR2 (Catalog # FAB151C, filled histogram) or PerCP-conjugated isotype control (Catalog # IC0041C, open histogram).

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 10⁶ cells/mL and 25 µL of cells (1 x 10⁵) 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/10⁵ 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 10⁵ cells) or 50 µL of packed whole blood to a 5 mL tube.
- 3) Add 10 µL of PerCP-conjugated CCR2 reagent.
- 4) Incubate for 30 - 45 minutes at 2° - 8° C.
- 5) Following this incubation, remove unreacted CCR2 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 this analysis, cells in a separate tube should be treated with PerCP-labeled mouse IgG_{2B} antibody.

This procedure may need modification, depending upon final utilization.

Background Information

CCR2 is a G-protein linked seven transmembrane domain spanning chemokine receptor that preferentially binds monocyte chemoattractant proteins-1 and -3 (MCP-1 and MCP-3).^{1,2} Two isoforms of this receptor (CCR2A and CCR2B) are expressed on cell surfaces as a result of alternate splicing from the same gene.¹ These two CCR2 variants differ only at their intracellular carboxyl terminals, with the CCR2A form possessing 14 additional amino acids. This may provide a mechanism by which cells responding to similar extracellular ligands can activate different intracellular second messengers. Cells that respond to the action of MCP-1 and therefore are likely to express CCR2 receptors include monocytes, T cells, NK cells, basophils, mast cells and dendritic cells.^{3,4} A recent report suggests that B cells may also express CCR2 receptors.⁵ The recognition that a variety of chemokine receptors, including CCR2, can serve as HIV fusion co-factors,⁶ and as facilitators of T cell recruitment during inflammation,⁷ makes chemokine receptor monitoring an important exercise in elucidating the HIV infection process and the regulation of inflammatory reactions.

References

1. Charo, I.F. *et al.* (1994) *Proc. Natl. Acad. Sci USA* **91**:2752.
2. Myers, S.J. *et al.* (1995) *J. Biol. Chem.* **270**:5786.
3. Jiang, Y. *et al.* (1992) *J. Immunol.* **148**:2423.
4. Locati, M. *et al.* (1994) *J. Biol. Chem.* **269**:4746.
5. Frade, J.M.R. *et al.* (1997) *J. Immunol.* **159**:5576.
6. Doranz, B.J. *et al.* (1996) *Cell* **85**:1149.
7. Loetscher, P. *et al.* (1996) *J. Exp. Med.* **184**:569.

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.