Monoclonal Anti-human CXCR2/IL-8 RB-PerCP

Catalog Number: FAB331C
Lot Number: AAQU01
100 Tests

Reagents Provided

Peridinin-Chlorophyll-Protein-Complex (PerCP)-conjugated mouse monoclonal anti-human CXCR2/IL-8 RB: Supplied as 25 μg of antibody in 1 mL PBS containing 0.1% sodium azide.

Clone #: 48311

Isotype: mouse IgG_{2A}

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

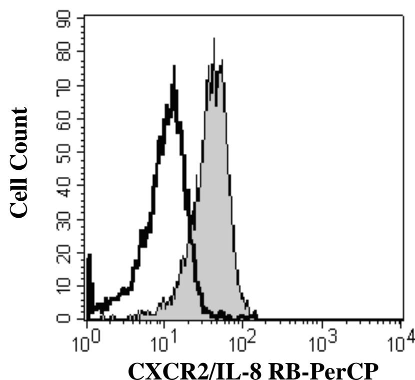
Designed to quantitatively determine the percentage of cells bearing CXCR2/IL-8 RB within a population and qualitatively determine the density of CXCR2/IL-8 RB on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the PerCP-labeled monoclonal antibody, which binds to cells expressing CXCR2/IL-8 RB. Unbound PerCP-conjugated antibody is then washed from the cells. Cells expressing CXCR2/IL-8 RB are fluorescently stained, with the intensity of staining directly proportional to the density of expression of CXCR2/IL-8 RB. Cell surface expression of CXCR2/IL-8 RB 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 CXCR2/IL-8 RB:
Use as is; no preparation necessary.



Human neutrophils were stained with PerCP-conjugated anti-human CXCR2/IL-8 RB (Catalog # FAB331C, filled histogram) or isotype control (Catalog # IC003C, open histogram).

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2008/07/24

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 10^6 cells/mL and 25 μL of cells (1 x 10^5) 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/10^5 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 10^5 cells) or 50 μL of packed whole blood to a 5 mL tube.
3) Add 10 μL of PerCP-conjugated CXCR2/IL-8 RB reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unreacted CXCR2/IL-8 RB 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_{2A} antibody.

This procedure may need modification, depending upon final utilization.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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1-800-343-7475

Background Information

The human C-X-C chemokine IL-8 is a potent neutrophil chemotactic and activating factor.[1] Two distinct cell surface receptors can interact with the IL-8 molecule. These two structures are known as IL-8 RA (type I or CXCR1) and IL-8 RB (type II or CXCR2).[2,3] The two receptors share 77% amino acid homology and are both members of the rhodopsin superfamily of G protein-linked receptors that span the cell membrane with seven hydrophobic transmembrane domains. Of the two IL-8 receptors, only CXCR1 (IL-8 RA) exclusively binds IL-8.[4] A third IL-8 binding structure, known as the Duffy blood group antigen, has been identified on red blood cells.[5] CXCL1 expression has been documented on neutrophils, monocytes and a small population of T cells.[2] The CXCR2 receptor has the ability to bind a variety of chemokines, including IL-8, GROα, β and γ, NAP-2 and ENA-78.

References

1. Oppenheim, J.J. (1991) Annu. Rev. Immunol. **9**:617.
2. Holmes, W.E. *et al*. (1991) Science **253**:1278.
3. Murphy, P.M. and H.L. Tiffany (1991) Science **253**:1280.
4. LaRosa, G.J. *et al.* (1992) J. Biol. Chem. **267**:25402.
5. Horuk, R. *et al.* (1993) Biochemisry **32**:5733.

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.