Monoclonal Anti-human CCR8-Fluorescein

Catalog Number: FAB1429F
Lot Number: LCV02
100 Tests

Reagent Information

Carboxyfluorescein-conjugated rat monoclonal anti-human CCR8: Supplied as 100 μg of antibody in 1 mL PBS containing 0.1% sodium azide.

Clone #: 191704

Ig Class: rat IgG_{2B}

Additional Reagents Required

- 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 expressing the chemokine receptor CCR8 within a population and qualitatively determine the density of CCR8 on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the fluorescein-labeled monoclonal antibody that binds to the cells expressing human CCR8. Unbound fluorescein-conjugated antibody is then washed from the cells. Cells expressing CCR8 are fluorescently stained, with the intensity of staining directly proportional to the density of CCR8. Cell surface expression of CCR8 is determined by flow cytometric analysis using 488 nm wavelength laser excitation.

Reagent Preparation

Carboxyfluorescein-conjugated rat anti-human CCR8: Use as is; no preparation is necessary.

Sample Preparation

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

Note that cells that express high levels of Ig FcR (CD16, CD32 and/or CD64) may exhibit higher background staining. We recommend that these cells be first treated with FcR blocking reagents (such as excess IgG or with antibodies specific for the FcR structures).

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), as described above, 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 4×10^6 cells/mL and 25 μL of cells (1×10^5) are transferred to a 5 mL tube for staining.

Note: Adherent cell lines may require pretreatment with 0.5 mM EDTA to facilitate removal from substrate. Cells that require trypsinization to enable removal from substrate 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 to be used for staining with the antibody may be first Fc-blocked by treatment with 1 μg of mouse or human IgG/10^5 cells for 15 minutes at room temperature. Do not wash excess blocking IgG from this reaction.
2) Transfer 25 μL of the Fc-blocked cells (1×10^5 cells) or 50 μL of packed whole blood to a 5 mL tube.
3) Add 10 μL of fluorescein-conjugated anti-human CCR8 reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unreacted anti-CCR8 reagent by washing (described above) the cells twice in 4 mL of the same PBS buffer (*note that whole blood will require a RBC lysis step at this point using any commercially available lysing reagent, such as R&D Systems Human Erythrocyte Lysing Kit, Catalog # WL1000*).
6) Resuspend the cells in 200 - 400 μL of PBS buffer for final flow cytometric analysis.
7) As a control for analysis, cells in a separate tube should be treated with fluorescein-labeled rat IgG_{2B} antibody.

This procedure may need modification, depending upon final utilization.

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Background Information

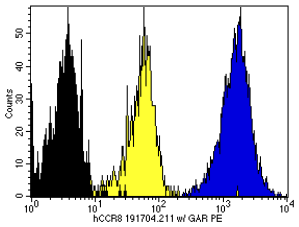
The human CCR8 chemokine receptor is a member of the rhodopsin superfamily of G-linked receptors that span the cell membrane with seven hydrophobic transmembrane domains. CCR8, also known as TER1, ChemR1 and CKR-L1, is the receptor for the chemokine CCL1 (I-309) (1, 2). Three virally encoded ligands, vMIP-I, vMIP-II and vMCC-I, can also interact with the CCR8 receptor (3). CCR8 expression has been reported on peripheral blood monocytes, thymocytes and on Th2 polarized T cells (2, 4 - 7).

This monoclonal to human CCR8 has been successfully used to stain monocytes, appropriately transfected cells and tumor cell lines such as HL-60 and THP-1 (in-house data).

References

1. Roos, R.S. *et al*. (1997) J. Biol. Chem. **272**:17251.
2. Tiffany, H.L. *et al*. (1997) J. Exp. Med. **186**:165.
3. Dairaghi, D.J. *et al*. (1999) J. Biol. Chem. **274**:21569.
4. Napolitano, M. *et al*. (1996) J. Immunol. **157**:2759.
5. Zaballos, A. *et al*. (1996) Biochem. Biophys. Res. Commun. **227**:846.
6. D'Ambrosio, D. *et al*. (1998) J. Immunol. **161**:111.
7. Zingoni, A. *et al*. (1998) J. Immunol. **161**:547.

Figure 1



The reactivity of anti-human CCR8 monoclonal antibody (clone # 191704) on two cell types is demonstrated in Figure 1. The light filled histogram (middle) represents the monoclonal antibody reacting with human peripheral blood monocytes. The dark filled histogram on the right demonstrates the reactivity of the monoclonal antibody with the human THP-1 cell line. Isotype control staining is shown in the dark filled histogram positioned on the left.

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.