

Monoclonal Anti-human CCR9-Allophycocyanin

Catalog Number: FAB1791A

Lot Number: LOX05

100 Tests

Reagent Information

Allophycocyanin (APC)-conjugated mouse monoclonal anti-human CCR9: Supplied as 10 µg of antibody in 1 mL PBS containing 0.1% sodium azide.

Clone #: 248621

Ig Class: mouse IgG_{2A}

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

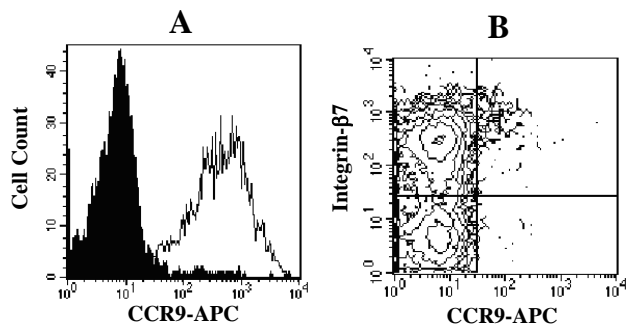
Designed to quantitatively determine the percentage of cells bearing the cell surface receptor CCR9 within a population and qualitatively determine the density of this receptor on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the APC-labeled monoclonal antibody, which binds to the cells expressing CCR9. Unbound APC-conjugated antibody is then washed from the cells. Cells expressing CCR9 are fluorescently stained, with the intensity of staining directly proportional to the density of CCR9. Cell surface expression of CCR9 is determined by flow cytometric analysis using 620 - 650 nm wavelength laser excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 660 - 670 nm.

Reagent Preparation

APC-conjugated mouse anti-human CCR9: Use as is; no preparation is necessary.



A. Molt-4 cells stained with anti-human CCR9-APC (Catalog # FAB1791A, open histogram) or isotype control (Catalog # IC003A, filled histogram).

B. Human peripheral blood lymphocytes stained with anti-Integrin β7-PE and anti-human CCR9-APC.

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) 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. Whole blood cells 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), 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 x 10⁶ cells/mL and 25 µL of cells (1 x 10⁵) 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/10⁵ 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 x 10⁵ cells) or 50 µL of packed whole blood to a 5 mL tube.
- 3) Add 10 µL of APC-conjugated anti-CCR9 reagent.
- 4) Incubate for 30 - 45 minutes at 2° - 8° C.
- 5) Following this incubation, remove unreacted anti-CCR9 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 APC-labeled mouse IgG_{2A} antibody.

This procedure may need to be modified, depending upon final utilization.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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Background Information

Human CCR9, previously known as GPR-9-6 (1), is a member of the large family of seven transmembrane spanning G-protein coupled receptors (2). Human CCR9 exists as 2 isoforms, CCR9A and CCR9B (3). The "A" isoform contains an additional 12 amino acids at the N-terminus, and appears to be expressed in thymocytes, lymphoid cell lines and PBMC at a 10-fold greater level than CCR9B (3). Studies have shown expression of CCR9 mRNA in both mature and immature thymocytes with double positive CD4⁺/CD8⁺ thymocytes expressing higher levels than single positive, mature thymocytes (3 - 5). Low levels of CCR9 mRNA have been observed in peripheral blood lymphocytes and in some T cell lines such as MOLT-4, MOLT-13 and SUP-T1 (3, 5). Low levels of cell surface CCR9 have been demonstrated on CD19⁺ B cells, and on CD4⁺ and CD8⁺ memory T cells (5). More specifically, CCR9 expression identifies $\alpha 4\beta 7^+$ CD4⁺ and CD8⁺ memory T cells that home to the gut (5). Alternatively, lack of CCR9 expression and the presence of CCR4 may help define another subset of memory CD4⁺ T cells that are CLA⁺ (cutaneous leukocyte-associated antigen) and appear to home to lymphoid organs other than the gut (5). CCR9 is the receptor for the chemokine CCL25/TECK (thymus expressed chemokine) that is expressed preferentially in fetal spleen, fetal and adult small intestine, and adult thymus (3 - 6). The chemotactic effects of TECK for activated macrophages, dendritic cells and thymocytes (4, 6) suggest a role for TECK and CCR9 in tissue-specific lymphocyte migration. This monoclonal antibody was selected for its ability to recognize a subset of peripheral blood integrin $\beta 7^+$ T cells (5, in house data).

References

1. Zaballo, A. *et al.* (1999) *J. Immunol.* **162**:5671.
2. David, J. and F. Mortari (2000) *Clin. Appl. Immunol. Rev.* **1**:105.
3. Yu, C.R. *et al.* (2000) *J. Immunol.* **164**:1293.
4. Youn, B.S. *et al.* (1999) *Blood* **94**:2533.
5. Zabel, B.A. *et al.* (1999) *J. Exp. Med.* **190**:1241.
6. Vicari, A.P. *et al.* (1997) *Immunity* **7**:291.

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.