

Monoclonal Anti-human CXCR7/RDC-1-Phycoerythrin

Catalog Number: FAB42271P

Lot Numbers: AAEL01

AAEL02

100 Tests

Reagents Provided

Phycoerythrin (PE)-conjugated mouse monoclonal anti-human CXCR7/RDC-1: Supplied as 25 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 358426

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 CXCR7/RDC-1 within a population and qualitatively determine the density of CXCR7/RDC-1 on cell surfaces by flow cytometry.

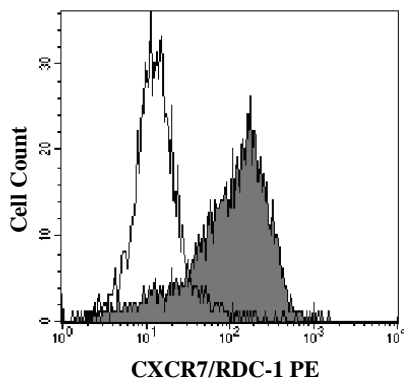
Principle of the Test

Washed cells are incubated with the phycoerythrin-labeled monoclonal antibody, which binds to cells expressing CXCR7/RDC-1. Unbound phycoerythrin-conjugated antibody is then washed from the cells. Cells expressing CXCR7/RDC-1 are fluorescently stained, with the intensity of staining directly proportional to the density of expression of CXCR7/RDC-1. Cell surface expression of CXCR7/RDC-1 is determined by flow cytometric analysis using 488 nm wavelength laser excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 565 - 605 nm.

Reagent Preparation

Phycoerythrin-conjugated mouse anti-human

CXCR7/RDC-1: Use as is; no preparation necessary.



Human monocytes were stained with PE-conjugated anti-human CXCR7/RDC-1 (Catalog # FAB42271P, filled histogram) or isotype control (Catalog # IC003P, 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) by centrifugation at 500 x g for 5 minutes. Transfer 50 µL of packed cells 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), 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) 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 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 PE-conjugated CXCR7/RDC-1 reagent.
- 4) Incubate for 30 - 45 minutes at 2° - 8° C.
- 5) Following this incubation, remove unreacted CXCR7/RDC-1 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 final flow cytometric analysis.
- 7) As a control for analysis, cells in a separate tube should be treated with PE-labeled mouse IgG_{2A} antibody.

This procedure may need modification, depending upon final utilization.

Background Information

The G protein-coupled receptor, RDC1, belongs to a subgroup of chemokine receptors and has been designated CXCR7. CXCR7 can bind with high-affinity to CXCL12/SDF-1 and CXCL11/I-TAC. It is also a co-receptor for several HIV and SIV strains. In their N-termini and extracellular loops 1, 2, and 3, human and mouse CXCR7 share 84%, 100%, 96% and 86% amino acid (aa) sequence identity, respectively. Reports of mRNA levels and/or protein expression (as assessed using anti-CXCR7, clone 9C4) (J. Biol. Chem. 2005, **280**(42):35760, J. Immunol. 2006, **176**(4):2197) indicate that CXCR7 occurs on a wide variety of tissues and cells including monocytes, B cells, T cells and mature dendritic cells. In contrast, based on ligand binding analysis and receptor level (as assessed using anti-CXCR7, clone 11G8), surface expression of CXCR7 was reported to be restricted to tumor cells, activated endothelial cells, fetal liver cells, and few other cell types (J. Exp. Med. 2006, **203**(9):2201). The basis of these inconsistent observations is not known but may be attributed to cell context and the use of different antibodies that may recognize different epitopes.

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.