Monoclonal Anti-human CXCR4-Biotin Sampler Pack

Catalog Number: FABSP2B
100 Total Tests

Reagent Information

Biotin-conjugated mouse monoclonal anti-human CXCR4: Contains 250 µL of ready to use biotin-labeled antibody (sufficient for 25 tests) clone # 12G5 (Catalog # FAB170B). Mouse IgG_{2A} isotype.

Biotin-conjugated mouse monoclonal anti-human CXCR4: Contains 250 µL of ready to use biotin-labeled antibody (sufficient for 25 tests) clone # 44708 (Catalog # FAB171B). Mouse IgG_{2A} isotype.

Biotin-conjugated mouse monoclonal anti-human CXCR4: Contains 250 µL of ready to use biotin-labeled antibody (sufficient for 25 tests) clone # 44716 (Catalog # FAB172B). Mouse IgG_{2B} isotype.

Biotin-conjugated mouse monoclonal anti-human CXCR4: Contains 250 µL of ready to use biotin-labeled antibody (sufficient for 25 tests) clone # 44717 (Catalog # FAB173B). Mouse IgG_{2B} isotype.

Storage: 2 - 8° C

Additional Reagents Required

- PBS (Dulbecco's PBS)
- BSA

Intended Use

Designed to quantitatively determine the percentage of cells bearing the cell surface receptor CXCR4 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 biotin-labeled monoclonal antibody, which binds to the cells expressing the CXCR4 receptor. Unbound biotin-conjugated antibody is then washed from the cells. Following the use of a secondary developing reagent like avidin-FITC or streptavidin-PE, cells expressing the CXCR4 structure are fluorescently stained, with the intensity of staining directly proportional to the density of the CXCR4. Cell surface expression of the CXCR4 is determined by flow cytometric analysis using 488 nm wavelength laser excitation.

Reagent Preparation

Biotin-conjugated mouse anti-human CXCR4: Use as is; no preparation is necessary.

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 4×10^6 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 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 biotin-conjugated anti-CXCR4 reagent.
4) Incubate for 30 - 45 minutes at 2 - 8° C.
5) Following this incubation, remove unreacted anti-CXCR4 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 Whole Blood Lysing Kit, Cat # WL1000*).
6) Resuspend cells in approximately 100 µL of PBS buffer and then add 10 µL of either avidin-FITC (10 µg/mL stock) or streptavidin-PE (5 µg/mL stock) and allow the reaction to develop for 30 minutes at 2 - 8° C.
7) Finally, wash the cells as step 5 above and resuspend the cells in 200 - 400 µL of PBS buffer for final flow cytometric analysis.
8) As a control for analysis, cells in a separate tube should be treated with the appropriate biotin-labeled isotype control.

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

R&D Systems, Inc.
1-800-343-7475

6/04

Background Information

CXCR4 (fusin or LESTR) is a G-protein linked seven transmembrane spanning chemokine receptor that binds stromal cell-derived factor-1 (SDF-1) also known as CXCL12) (1, 2). Additionally, CXCR4 has been found to act as a co-factor for the entry into cells of the T-cell tropic HIV-1 and HIV-2 virus (3, 4). A monoclonal antibody (clone 12G5) to the CXCR4 structure was originally developed by Enders *et al.* (4) and shown by flow cytometry applications to react with CXCR4 expressed on a variety of human cell lines, including Sup-T1, Hut-78, Molt4, CEMss, Daudi and Hela, as well as with peripheral blood lymphocytes (4). This monoclonal can neutralize infection and inhibit syncytium formation induced by the HIV virus (4). **FAB170B** reacts with a region of the CXCR4 receptor that appears to require the presence of both extracellular loops 1 and 2, with the first extracellular loop playing a more critical role in this study (5). CXCR4 appears to exist as antigenically distinct conformations on various cell lines and primary cells. To address this issue R&D Systems generated a panel of monoclonal antibodies to the CXCR4 receptor whose reactivities are best summarized in a study by Baribaud, F. *et al.* (6). All three R&D Systems generated monoclonals, **FAB171B**, **FAB172B** and **FAB173B,** react preferentially with the second extracellular loop (third extracellular domain) of the CXCR4 receptor. Note that FAB173B was shown to be superior to other CXCR4 monoclonals in recognizing various conformations of CXCR4 (6). Additionally **FAB173B** recognizes feline CXCR4 (6).

References

1. Bleul, C.C. *et al.* (1996) Nature **382**:829.
2. Oberlin, E. *et al.* (1996) Nature **382**:833.
3. Feng, Y. *et al.* (1996) Science **272**:872.
4. Endres, M.J. *et al.* (1996) Cell **87**:745.
5. Lu, Z.H. *et al.* (1997) Proc. Natl. Acad. Sci. USA **94**:6426.
6. Baribaud, F. et al. (2001) J. Virol. **75**:8957.

Note: 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.