Monoclonal
Anti-human Integrin α3/CD49c-Phycoerythrin

Catalog Number: FAB1345P
Lot Number: AAKL01
100 Tests

Reagents Provided

Phycoerythrin (PE)-conjugated mouse monoclonal anti-human Integrin α3/CD49c: Supplied as 25 µg of antibody in 1 mL PBS containing 0.1% sodium azide.

Clone #: IA3

Isotype: mouse IgG_1

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

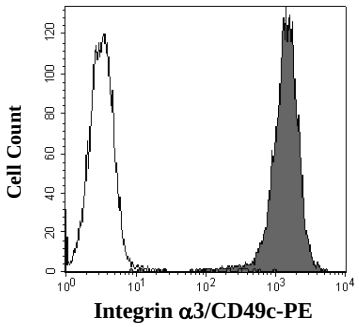
Designed to quantitatively determine the percentage of cells bearing Integrin α3/CD49c within a population and qualitatively determine the density of Integrin α3/CD49c on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the phycoerythrin-labeled monoclonal antibody, which binds to cells expressing Integrin α3/CD49c. Unbound phycoerythrin-conjugated antibody is then washed from the cells. Cells expressing Integrin α3/CD49c are fluorescently stained, with the intensity of staining directly proportional to the density of expression of Integrin α3/CD49c. Cell surface expression of Integrin α3/CD49c 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 Integrin α3/CD49c: Use as is; no preparation necessary.



HT-1080 cells were stained with PE-conjugated anti-human Integrin α3/CD49c (Catalog # FAB1345P, filled histogram) or isotype control (Catalog # IC002P, 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 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 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/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 PE-conjugated Integrin α3/CD49c reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unreacted Integrin α3/CD49c 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_1 antibody.

This procedure may need modification, depending upon final utilization.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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

2/08

Background Information

The α3 subunit, also known as CD49c and VLA-3 α subunit, forms a non-covalent heterodimer with the Integrin β1 subunit (CD29). Integrin α3β1 is a receptor for laminin, fibronectin, collagen, epiligrin, and thrombospondin.

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.