Monoclonal Anti-human CD58/LFA-3-Phycoerythrin

Catalog Number: FAB1689P
Lot Number: LSR02
100 Tests

Reagent Information

Phycoerythrin (PE)-conjugated mouse monoclonal anti-human CD58: Supplied as 25 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 248310

Isotype: mouse 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

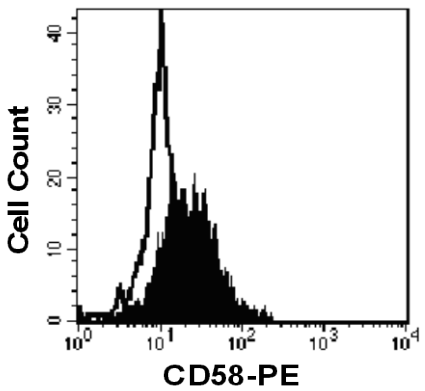
Designed to quantitatively determine the percentage of cells bearing CD58 within a population and qualitatively determine the density of CD58 on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the PE-labeled monoclonal antibody, which binds to cells expressing CD58. Unbound PE-conjugated antibody is then washed from the cells. Cells expressing CD58 are fluorescently stained, with the intensity of staining directly proportional to the density of expression of CD58. Cell surface expression of CD58 is determined by flow cytometric analysis using 488 nm wavelength laser excitation.

Reagent Preparation

PE-conjugated mouse anti-human CD58: Use as is; no preparation necessary.



Human peripheral blood monocytes stained with PE-conjugated anti-human CD58 antibody (Catalog # FAB1689P, filled histogram) or PE-conjugated isotype control antibody (Catalog # IC0041P, 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 4×10^6 cells/mL and 25 μL of cells (1×10^5) 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 (up to 1×10^6 cells) or 50 μL of packed whole blood to a 5 mL tube.
3) Add 10 μL of PE-conjugated anti-CD58 reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unreacted anti-CD58 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_{2B} antibody.

This procedure may need modification, depending upon final utilization.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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Background Information

CD58, also known as lymphocyte function-associated antigen- (LFA-3), is a 210 amino acid protein that belongs to the CD2 family of the immunoglobulin superfamily (1, 2). CD58 is widely expressed on hematopoietic and non-hematopoietic tissues including leukocytes, erythrocytes, endothelial cells, epithelial cells and fibroblasts of human origin (2 - 4). Two isoforms of CD58, a transmembrane form and a GPI-linked form are equally expressed on all nucleated cells (4, 5), whereas only the GPI-anchored form is expressed on erythrocytes (6). No mouse or rat homologue of CD58 is yet identified. The only known ligand for CD58 is CD2 (1, 2). Ligation of CD2 by CD58 mediates T cell adhesion, T cell activation, T cell cytokine production, and T cell and NK cell cytolytic activity (7 - 10). CD2 ligation on dendritic cells results in an increase in MHC class II expression, as well as CD40, CD80, CD86, CD58 and CCR7 expression (11). CD58 binding to CD2 also induces dendritic cells to secrete IL-1β and IL-12 (11).

References

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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.