Monoclonal Anti-human CD38-Fluorescein

Catalog Number: FAB2404F
Lot Number: LOB03
100 Tests

Reagent Information

Carboxyfluorescein (CFS)-conjugated mouse monoclonal anti-human CD38: Supplied as 25 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 240742

Isotype: 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

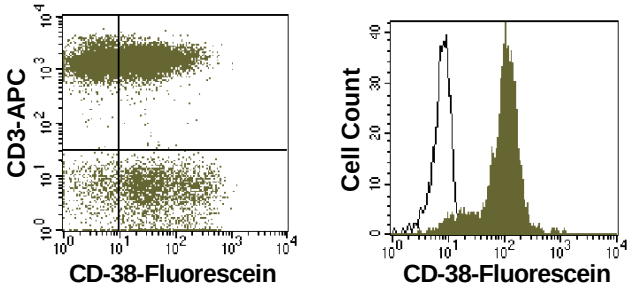
Designed to quantitatively determine the percentage of cells bearing CD38 within a population and qualitatively determine the density of CD38 on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the fluorescein-labeled monoclonal antibody, which binds to cells expressing CD38. Unbound fluorescein-conjugated antibody is then washed from the cells. Cells expressing CD38 are fluorescently stained, with the intensity of staining directly proportional to the density of expression of CD38. Cell surface expression of CD38 is determined by flow cytometric analysis using 488 nm wavelength laser excitation.

Reagent Preparation

Fluorescein-conjugated mouse anti-human CD38: Use as is; no preparation necessary.



Left Panel: Whole blood lymphocytes stained with anti-human CD38-Fluorescein (Catalog # FAB2404F) and anti-human-CD3-APC (Catalog # FAB100A). Right Panel: Whole blood monocytes stained with anti-human CD38-Fluorescein (Catalog # FAB2404F, filled histogram) or with isotype control antibody (Catalog # IC003F, 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 fluorescein-conjugated anti-CD38 reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unreacted anti-CD38 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 fluorescein-labeled mouse IgG_{2A} antibody.

This procedure may need modification, depending upon final utilization.

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Background Information

The CD38 cell surface antigen is a 46 kDa type II transmembrane glycoprotein with a short, N-terminal cytoplasmic tail (1, 2). CD38 was originally described as the T10 antigen expressed on thymocytes and leukemic early T cells (2, 3). The human CD38 molecule was cloned from a lymphocyte cDNA library transiently expressed in COS cells (3). In addition to its expression on thymocytes, CD38 is expressed on T cells, NK cells, monocytes, B cells and plasma cells (4, 5). CD38 is also shed from the cell surface and exists as a soluble molecule (6).

Functionally, CD38 is very complex. Signaling through CD38 has a mitogenic effect (7) and physically plays a role in adhesion by binding to its ligand PECAM-1/CD31 (8). CD38 also functions as an ectoenzyme exhibiting a variety of activities. CD38 catalyzes both the formation and hydrolysis of cyclic ADP ribose (7, 9) and functions as an NAD^+-glycohydrolase (10). Clinically, expression of CD38 may play a role in diabetes (11) and is thought to have prognostic value in HIV (12) and chronic lymphocytic leukemia (13, 14).

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.