Monoclonal Anti-human ICAM-3/CD50-Fluorescein

Catalog Number: BBA35
Lot Number: LAF01
100 Tests

Reagents Provided

Carboxyfluorescein-conjugated mouse monoclonal anti-human ICAM-3/CD50: Supplied as 25 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: Cal 3.10 (2A5)

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

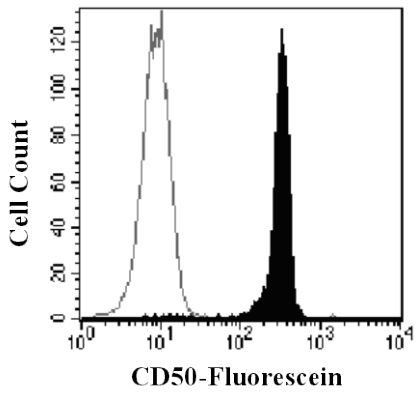
Designed to quantitatively determine the percentage of cells bearing ICAM-3/CD50 within a population and qualitatively determine the density of ICAM-3/CD50 on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the fluorescein-labeled monoclonal antibody, which binds to cells expressing ICAM-3/CD50. Unbound fluorescein-conjugated antibody is then washed from the cells. Cells expressing ICAM-3/CD50 are fluorescently stained, with the intensity of staining directly proportional to the density of expression of ICAM-3/CD50. Cell surface expression of ICAM-3/CD50 is determined by flow cytometric analysis using 488 nm wavelength laser excitation.

Reagent Preparation

Fluorescein-conjugated mouse anti-human ICAM-3/CD50: Use as is; no preparation necessary.



Human peripheral blood neutrophils stained with Fluorescein-conjugated anti-human ICAM-3 (Catalog # BBA35, filled histogram) or with Fluorescein-conjugated isotype control (Catalog # IC002F, 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 (1×10^5 cells) or 50 μL of packed whole blood to a 5 mL tube.
3) Add 10 μL of fluorescein-conjugated anti-ICAM-3 reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unreacted anti-ICAM-3 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_1 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

Intracellular Molecule-3 (ICAM-3/CD50) is a member of the immunoglubulin supergene family type I protein of 518 amino acids (1). Its extracellular region of 456 amino acids consists of five immunoglobulin-like domains with 15 potential N-glycosylation sites (1). ICAM-3 is capable to interacting with such molecules as LFA-1, Integrin α-d/β2 and DC-SIGN as a result can regulate the cellular adhesion interactions associated with the molecules in question (2 - 4). Additional functions associated with ICAM-3/CD50 include regulation of co-stimulatory signals in immune responses, regulation of cellular morphology and regulation of cell motility during chemotactic events (5 - 7). ICAM-3/CD50 is expressed all human leukocytes as well as on epidermal dendritic Langerhans cells (8).

References

1. Fawcett, J. *et al*. (1992) Nature **360**:481.
2. Campanero, M.R. *et al*. (1993) J. Cell Biol. **123**:1007.
3. Van der Vieren, *et al*. (1995) Immunity **3**:683.
4. Geijtenbeek, T.B.H. *et al*. (2000) Nature **1**:353.
5. de Fougerolles, A.R. and T.A. Springer (1994) J. Exp. Med. **179**:619.
6. Hernandez-Caselles, T. *et al*. (1993) Eur. J. Immunol. **23**:2799.
7. del Pozo, M.A. *et al*. (1995) J. Cell Biol. **131**:495.
8. Acevedo, A. *et al*. (1993) Am. J. Pathol. **143**:774.

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.