Monoclonal Anti-human VCAM-1-Fluorescein (CD106)

Catalog Number: BBA22
Lot Number: LAC07
100 Tests

Reagents Provided

Fluorescein-conjugated mouse monoclonal anti-human VCAM-1/CD106: Supplied as 50 µg of antibody in 1 mL PBS containing 0.1% sodium azide.

Clone #: BBIG-V3 (IE10)

Isotype: mouse IgG_{2A}

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

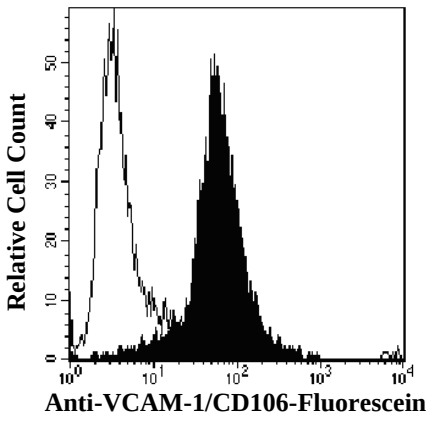
Designed to quantitatively determine the percentage of cells bearing VCAM-1 (CD106) within a population and qualitatively determine the density on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the fluorescein-labeled monoclonal antibody that binds to cells expressing VCAM-1. Unbound fluorescein-conjugated antibody is then washed from the cells. Cells expressing VCAM-1 are fluorescently stained, with the intensity of staining directly proportional to the density of VCAM-1. Cell surface expression of VCAM-1 is determined by flow cytometric analysis using 488 nm wavelength laser excitation.

Reagent Preparation

Fluorescein-conjugated mouse anti-human VCAM-1 (CD106): Use as is; no preparation necessary.



Anti-human VCAM-1/CD106 staining of HUT-78 cells using either isotype control (Catalog # IC003F; open histogram) or with anti-VCAM-1-Fluorescein (Catalog # BBA22; filled 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. 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 x 10^6 cells/mL and 25 µL of cells (1 x 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 x 10^5 cells) or 50 µL of packed whole blood to a 5 mL tube.
3) Add 10 µL of fluorescein-conjugated anti-VCAM-1 reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unreacted anti-VCAM-1 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, 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 to be modified, depending upon final utilization.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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1-800-343-7475

Background Information

Human Vascular Cell Adhesion Molecule-1 (VCAM-1/CD106) is a 100 - 110 kDa, 715 amino acid, type I transmembrane glycoprotein (1 - 3). A number of variants of human VCAM-1 occur as a result of alternate gene splicing (1). Moreover, a soluble form of VCAM-1 has been identified in culture supernatants (4), blood (5 - 7) and cerebrospinal fluid (7, 8). Various proteases, including MMPs, neutrophil elastase, and cathepsin B have been implicated in the shedding of transmembrane VCAM-1 (9, 10). VCAM-1 is expressed constitutively on non-vascular cells including: dendritic cells in lymphoid tissues and skin (11), macrophages (11), fibroblasts (12), melanoma cells (11), smooth muscle cells (13), adult satellite muscle cells (14), bone marrow stromal cells (15), chondrocytes (16), mesothelium (17), renal tubular epithelium and mesangium (18), embryonic myoblasts and myotubules (14), neurons (19) and choroid plexus epithelium (20). VCAM-1 expression on vascular endothelium can be induced by a number of inflammatory stimuli (11).

Functionally, VCAM-1 mediates cell adhesion and signal transduction by binding to its ligands/counter-receptors. Ligands for VCAM-1 are the integrins $\alpha_4\beta_1$ (CD49d/CD29 or VLA4) and $\alpha_4\beta_7$ (11, 21 - 24), $\alpha_D\beta_2$ (25, 26), and $\alpha_9\beta_1$ (27). These VCAM-1 ligands are expressed on a variety of lymphoid cells. Accordingly, VCAM-1/VCAM-1 ligand interactions are key events in the rate and timing of leukocyte extravasation (11). Similarly, VCAM-1 mediates the adhesion of melanoma cells to endothelial cells and may play a role in metastasis (11). VCAM-1 expression on bone marrow stromal cells regulates T and B cell development as well as hematopoietic progenitor cell homing and trafficking (28 - 30). Other proposed roles for VCAM-1 include the regulation of osteoclastogenesis via a cell-to-cell contact mechanism (15), and the induction of sickle cell adherence to vascular endothelial cells during hypoxemia (31). Soluble VCAM-1 has been shown to mediate angiogenesis and is chemotactic for T lymphocytes and monocytes (11, 32).

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.